Guidance for Industry
Product Development Under the Animal Rule

DRAFT GUIDANCE

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Guidance for Industry
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U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

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Animal Rule

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This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the FDA staff responsible for implementing this guidance. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I.   INTRODUCTION

This guidance provides information and recommendations on drug and biological product[2] development when human efficacy studies are not ethical or feasible. The regulations that set forth the pathway for approval of these products under 21 CFR 314.600 (drugs) or 21 CFR 601.90 (biological products) are commonly referred to as the *Animal Rule*.

This draft guidance revises the 2009 draft guidance for industry *Animal Models – Essential Elements to Address Efficacy Under the Animal Rule.* While addressing the topics covered in the 2009 draft, this revision covers a broader scope of issues for drugs developed under the Animal Rule. For example, new sections have been added related specifically to study conduct and data quality and integrity[3] (section IV.B), development of vaccines (section VII.A), and development of cellular and gene therapies (section VII.B). There are new sections on FDA's general expectations for animal studies related to, for example, animals used in investigations, types of animal care interventions, and study reports (section IV). There is also a new section on FDA's general expectations regarding natural history studies (Appendix C).

This guidance does not address the following topics:

- The chemistry, manufacturing, and controls or nonclinical pharmacology and toxicology studies necessary for drug development

- Issues related to initial proof-of-concept studies

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[1] This guidance has been prepared by the Center for Drug Evaluation and Research (CDER) in cooperation with the Center for Biologics Evaluation and Research (CBER) at the Food and Drug Administration. The Office of Good Clinical Practice and the Office of Counterterrorism and Emerging Threats also provided input.

[2] As used in this guidance, all references to *drugs* include human drugs, therapeutic biological products, cellular and gene therapies, and vaccines, unless otherwise specified.

[3] In promulgating the Animal Rule, FDA stated that "…studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations…" (67 *Federal Register* 37988 at 37989, May 31, 2002). The good laboratory practice regulations (GLP), however, were developed as a quality system for nonclinical safety studies. FDA's current expectations are described in section IV.B.

35   • The details of study design and conduct for drug-specific animal efficacy studies or
36   human pharmacokinetics and/or safety studies

37   • Drug development in specific populations (e.g., children, geriatrics, and pregnant
38   women)

39   • The development of combination products

40   • Requirements for procurement of medical countermeasures by the Federal government
41   (e.g., Strategic National Stockpile (SNS)[4])

42   • The development of animal models for other purposes, such as for assessment of
43   toxicology
44

45   Information on FDA guidances is available on FDA's Web site.[5] In addition, FDA guidances
46   related to medical countermeasures for chemical, biological, radiological, and nuclear (CBRN)
47   agents can be accessed through FDA's Medical Countermeasures initiative (MCMi) Web site.[6]
48

49   FDA's guidance documents, including this guidance, do not establish legally enforceable
50   responsibilities. Instead, guidances describe the Agency's current thinking on a topic and should
51   be viewed only as recommendations, unless specific regulatory or statutory requirements are
52   cited. The use of the word *should* in Agency guidances means that something is suggested or
53   recommended, but not required.
54

55

56   **II.   THE ANIMAL RULE**
57

58   FDA's regulations concerning the approval[7] of new drugs when human efficacy studies are not
59   ethical or feasible are codified in 21 CFR 314.600 for drugs and 21 CFR 601.90 for biological
60   products. Approval under the Animal Rule can only be pursued if definitive human efficacy
61   studies cannot be conducted because it would be unethical and field trials have not been feasible.
62

63   The Animal Rule states that for drugs developed to ameliorate or prevent serious or life-
64   threatening conditions caused by exposure to lethal or permanently disabling toxic substances,
65   when human challenge studies would not be ethical to perform and field trials to study
66   effectiveness after accidental or intentional human exposure have not been feasible, FDA may
67   grant marketing approval based on adequate and well-controlled animal efficacy studies when
68   the results of those studies establish that the drug is reasonably likely to produce clinical benefit

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[4] Sponsors should discuss issues related to the SNS with the Department of Health and Human Services/Biomedical Advanced Research and Development Authority (HHS/BARDA) and the Centers for Disease Control and Prevention (CDC).

[5] FDA guidances are updated periodically. The most recent versions are available at
http://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

[6] The MCMi Web site is available at
http://www.fda.gov/emergencypreparedness/medicalcountermeasures/default.htm.

[7] As used in this guidance, the term *approval* refers to approval or licensure.

69 in humans. Drugs evaluated for efficacy under the Animal Rule should be evaluated for safety
70 under the existing requirements for establishing the safety of new drugs. The Animal Rule states
71 that FDA will rely on evidence from animal studies to provide substantial evidence[8] of
72 effectiveness only when all of the following four criteria, quoted below, are met:
73
74   1. There is a reasonably well-understood pathophysiological mechanism of the toxicity of
75   the substance and its prevention or substantial reduction by the product;
76
77   2. The effect is demonstrated in more than one animal species expected to react with a
78   response predictive for humans, unless the effect is demonstrated in a single animal
79   species that represents a sufficiently well-characterized animal model for predicting the
80   response in humans;
81
82   3. The animal study endpoint is clearly related to the desired benefit in humans, generally
83   the enhancement of survival or prevention of major morbidity; and
84
85   4. The data or information on the kinetics and pharmacodynamics of the product or other
86   relevant data or information, in animals and humans, allows selection of an effective dose
87   in humans.[9]
88
89 If all of these criteria are met, it is reasonable to expect the efficacy of the drug in animals to be a
90 reliable indicator of its effectiveness in humans.
91
92 The use of the Animal Rule as a regulatory pathway to approval is not confined to the
93 development of medical countermeasures for chemical, biological, radiological, or nuclear threat
94 agents. Drugs intended to ameliorate or prevent serious or life-threatening conditions due to
95 other toxic chemical, biological, radiological, or nuclear substances (e.g., emerging virus, snake
96 venom, industrial chemicals) may be eligible for development under the Animal Rule when it is
97 not ethical to conduct human challenge studies and when field trials to study effectiveness are
98 not feasible.
99
100 FDA will determine whether the previously noted criteria have been met and the Animal Rule
101 can be used. In general, the determination of whether it is ethical to conduct deliberate exposure
102 studies in humans is not difficult; however, the determination that human efficacy trials are not
103 feasible may be challenging. The feasibility issues to be considered will vary with the disease or
104 condition to be studied and may change over time. For example, there may be circumstances
105 that affect the feasibility of planning and execution of human efficacy studies for the disease or
106 condition, such as: (1) a low prevalence and/or incidence, (2) an unpredictable incidence rate

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[8] The term *substantial evidence* has been defined previously in the Federal Food, Drug, and Cosmetic Act (FD&C
Act) §505 (d) as "…evidence consisting of adequate and well-controlled investigations, including clinical
investigations, by experts qualified by scientific training and experience to evaluate the effectiveness of the drug
involved, on the basis of which it could fairly and responsibly be concluded by such experts that the drug will have
the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in
the labeling or proposed labeling thereof."

[9] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

107 from year to year, (3) an inability to predict geographic locations where outbreaks may occur, (4)
108 occurrences limited to areas lacking critical infrastructure, and/or (5) occurrences limited to areas
109 in which there is some extraordinary threat to subject or investigator safety. In addition, other
110 challenges, such as the inability to obtain permission from foreign governments, may preclude
111 the conduct of clinical investigations. Sponsors should provide FDA with a clear rationale to
112 support the use of the Animal Rule for the development of their drug before proceeding with
113 drug development.
114
115 With regard to establishing evidence of efficacy, the Animal Rule states: "In assessing the
116 sufficiency of animal data, the agency may take into account other data, including human data,
117 available to the agency."[10] For example, in 2012, levofloxacin received approval under the
118 Animal Rule for the treatment of plague due to *Yersinia pestis*. Efficacy was established in an
119 African green monkey model of pneumonic plague. Existing human data from levofloxacin's
120 prior approval for other respiratory infections (i.e., nosocomial and community-acquired
121 pneumonias) provided additional support for its likely effectiveness in the treatment of
122 pneumonic plague. When human efficacy data from a relevant indication may support the
123 approval of the Animal Rule-based indication, FDA encourages sponsors to evaluate the drug in
124 an indication for which obtaining human data is ethical and feasible using a traditional regulatory
125 pathway.[11]
126
127 Information obtained in a related human disease or condition may support the determination of
128 efficacy for the Animal Rule-based indication (e.g., if the drug targets a pathway in the
129 pathophysiological cascade that is common to both the disease or condition intended for
130 evaluation under the Animal Rule and a disease or condition for which clinical trials are
131 feasible). In addition, while data from other types of studies in animals and/or in vitro studies
132 may be supportive, adequate and well-controlled animal efficacy studies are required for
133 approval under the Animal Rule.
134
135 The Animal Rule specifies that the choice of species for the adequate and well-controlled
136 efficacy studies must be appropriate with regard to the disease or condition of interest and the
137 investigational drug.[12] There is no requirement for the use of a specific species. With respect to
138 each animal species selected by sponsors, the sponsors should provide scientific justification that
139 the animal species exhibits key characteristics of the human disease or condition when the
140 animal is exposed to the challenge agent.[13] In addition, the species should be selected based on
141 an understanding of the drug's mechanism of action, such that the drug's effect in the animal

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[10] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

[11] As stated in the preamble to the final rule (67 *Federal Register* 37988 at 37990, May 31, 2002), "…with anti-infective drug products, it would usually be expected that human data on safety and effectiveness for other indications may be available."

[12] See 21 CFR 314.610(a) for drugs and 21 CFR 601.91(a) for biological products.

[13] As used in this guidance, the term *challenge agent* refers to the substance used to cause the disease or condition in the animal studies, whereas the term *etiologic agent* refers to the substance causing the disease or condition in humans.

142 species is expected to be predictive of its effect in humans, and the ability to select an effective
143 dose and regimen for humans.
144
145 The number of animal species necessary to support approval of a drug under the Animal Rule
146 depends on the nature and clinical significance of any differences between the animal models[14]
147 and humans with regard to the essential elements as described in section V. Sponsors should
148 provide data or information to demonstrate that each animal model reflects key aspects of the
149 pathophysiology of the human disease or condition of interest and that the response to the
150 investigational drug in each animal model is likely to predict the response in humans.
151
152 FDA will evaluate the suitability of a proposed animal model on a case-by-case basis.
153 Generally, efficacy of the drug should be demonstrated in more than one animal species expected
154 to react with a response predictive for humans. In certain circumstances, studies in more than
155 two species may be necessary to model the relevant aspects of the human disease or condition
156 and response to the investigational drug. If the effect is demonstrated in a single species that
157 represents a sufficiently well-characterized animal model[15] for predicting the response in
158 humans, then the Animal Rule allows for approval based on substantial evidence of effectiveness
159 demonstrated in studies conducted in that species. The acceptability of using a single animal
160 species will require FDA review and agreement on the body of evidence supporting the adequacy
161 of the model. As discussed in the preamble to the final rule, the "…circumstances in which the
162 agency will rely on evidence from studies in one animal species to provide substantial evidence
163 of the effectiveness of these products in humans would generally be limited to situations where
164 the study model is sufficiently well-recognized so as to render studies in multiple species
165 unnecessary. In addition, other human data for the product could provide support for such
166 approvals."[16]
167
168 When available, data from the use of the investigational drug in humans with the disease or
169 condition may provide a link between the well-characterized animal model and the predictive
170 response in humans. For example, Cyanokit (hydroxocobalamin) was approved for the treatment
171 of cyanide poisoning under the Animal Rule on the basis of one adequate and well-controlled
172 efficacy study in dogs with supporting evidence in humans from uncontrolled trials using
173 hydroxocobalamin to treat cyanide poisoning from smoke inhalation, cyanide ingestion, or
174 cyanide inhalation. The adequate and well-controlled study in dogs was determined to be
175 predictive of the response in humans; thus, this dog model was accepted as a well-characterized
176 animal model.
177
178 When efficacy is demonstrated in a single animal species that represents a sufficiently well-
179 characterized animal model, it may be necessary to reproduce the efficacy findings in that same

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[14] For the purpose of this guidance, an *animal model* is defined as a specific combination of an animal species, challenge agent, and route of exposure that produces a disease process or pathological condition that in multiple important aspects corresponds to the human disease or condition of interest.

[15] A *well-characterized animal model* was defined as "meaning the model has been adequately evaluated for its responsiveness" in the preamble to the final rule (67 *Federal Register* 37988 at 37989, May 31, 2002).

[16] See 67 *Federal Register* 37988 at 37991, May 31, 2002.

180 animal model with a confirmatory study.[17] Ideally, the efficacy findings should be reproduced in
181 a study conducted at a different laboratory; however, use of the same laboratory may be
182 acceptable with justification. Supportive human data in a related non-Animal Rule based-
183 indication may negate the need for a confirmatory study.
184
185 There may be situations in which the application of the Animal Rule requires a more complex
186 development plan. For example, variola virus (the causative agent of smallpox) presents a
187 unique challenge because humans are the only known natural host, no animal species has been
188 found to have comparable susceptibility to variola virus, and naturally occurring smallpox has
189 been eradicated. Therefore, efficacy of investigational drugs developed to treat smallpox needs
190 to be studied using other orthopoxviruses in relevant animal species (e.g., monkeypox in
191 nonhuman primates, rabbitpox in rabbits, or ectromelia in mice). Depending on the strength of
192 the animal studies and other supporting evidence, the efficacy findings from such studies may
193 support approval of the drug against variola. As with all animal efficacy studies, FDA strongly
194 recommends that, in such situations, sponsors discuss the scientific approach under consideration
195 with the review division before initiating the animal studies.
196
197 Approval of a drug under the Animal Rule imposes three additional requirements, which are
198 summarized below (for greater detail, see 21 CFR 314.610(b) (1)-(3) for drugs and 21 CFR
199 601.91(b) (1)-(3) for biological products):
200
201   1. Postmarketing studies (e.g., field studies) to provide evaluation of safety and clinical
202   benefit if circumstances arise in which a study would be feasible and ethical (i.e., in the
203   event an emergency arises and the drug is used). A plan or approach to conducting such
204   a study must be included with the new drug application (NDA) or biologics license
205   application (BLA).
206
207   2. Restrictions to ensure safe use, if needed (e.g., restricting distribution to facilities or
208   health care practitioners with special training, requiring specified types of follow up, or
209   imposing record keeping requirements).
210
211   3. Information to be provided in the labeling to patient recipients that explains that for
212   ethical or feasibility reasons, the drug's approval was based on efficacy studies conducted
213   in animals alone. This drug labeling should also include all the other relevant
214   information required by FDA at the time of approval (e.g., directions for use,
215   contraindications, a description of any reasonably foreseeable risks, adverse reactions,
216   anticipated benefits, and drug interactions).[18] This information must be provided before
217   administration or dispensing, if possible.
218
219

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[17] As stated in the preamble to the final rule, "…the animal studies should be replicated or substantiated in each species as needed to ensure credible results…" (67 *Federal Register* 37988 at 37991, May 31, 2002).

[18] See 21 CFR 314.610(b)(3) for drugs and 21 CFR 601.91(b)(3) for biological products.

220 **III.   REGULATORY CONSIDERATIONS**
221
222   **A.   Drug Development Plan**
223
224 Obtaining the body of evidence necessary to support approval of a drug using the Animal Rule is
225 a complex and iterative process. FDA strongly encourages sponsors to establish early and
226 ongoing communications with the Agency. Sponsors also may wish to seek input from public
227 health officials and/or the military about the potential need for, and operational use of, the
228 investigational drug and discuss this with FDA. Developing a drug development plan will
229 support the discussion of important issues, including, but not limited to, the following:

230   • The proposed indication and whether a drug can be developed under the Animal Rule

231   • The design of the animal studies (e.g., incorporation of supportive care) as it relates to the
232   anticipated medical management in humans

233   • The development and/or selection of the animal models, including, when necessary, the
234   design of the natural history studies

235   • The results of the proof-of-concept studies

236   • The proposed methods for selecting an effective dose and regimen in humans

237   • The design of the adequate and well-controlled animal efficacy studies intended to
238   provide the primary evidence of effectiveness of the drug

239   • The proposed approach for ensuring the quality and integrity of data[19]

240   • The size and composition of the human safety database

241   • Plans or approaches for conducting the required postmarketing studies (e.g., field studies)
242   to demonstrate safety and clinical benefit

243   • Timelines and/or triggers for FDA feedback or meetings

244   • Eligibility for expedited development and review designation programs

245   • Additional issues critical to the sponsor's funding agencies[20]
246

247 Drug development is data-driven; any development plan should allow for modification or
248 refinement as data are gathered and analyzed and projections or expectations change. It is the
249 sponsor's responsibility to provide complete and accurate submissions. Sponsors should explain
250 any proposed deviations from the recommendations expressed in this guidance. The potential

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[19] In promulgating the Animal Rule, FDA stated that "…studies subject to this rule must be conducted in accordance with preexisting requirements under the good laboratory practices (21 CFR part 58) regulations…" (67 *Federal Register* 37988 at 37989, May 31, 2002). The good laboratory practice regulations (GLP), however, were developed as a quality system for nonclinical safety studies. FDA's current expectations are described in section IV.B.

[20] The product development plan required by funding agencies for medical countermeasures against chemical, biological, radiological, or nuclear agents may dictate certain proof-of-concept studies and an accelerated timeline for efficacy studies in animals. The sponsor's relationship with their funding agency is independent of their relationship with FDA.

251 impact of these deviations on the drug development program should be discussed with FDA
252 before the conduct of the relevant studies.
253
254 FDA strongly recommends that sponsors obtain Agency concurrence on the design of the
255 adequate and well-controlled animal efficacy studies because these substitute for the efficacy
256 trials in humans (see sections VI and X). Sponsors should allow adequate time for FDA review,
257 comment, and agreement before initiating these studies to ensure that the study design is
258 adequate to support the proposed indication.
259
260 The protocols for animal efficacy studies intended to provide primary evidence of effectiveness
261 are eligible for evaluation under special protocol assessment (SPA) provisions.[21,22] Before
262 submitting the SPA request, the sponsor should have FDA concurrence on the model proposed
263 for use in the efficacy study, including, but not limited to, the species, the details of the challenge
264 agent, the conditions of exposure, and the method that will be used to select an effective dose
265 and regimen in humans.
266
267 Drugs developed under the Animal Rule may be eligible for certain expedited development and
268 review designation programs,[23] such as Fast Track and Priority Review, or other FDA programs,
269 such as Orphan Drug Designation.[24] Sponsors requesting these designations should use
270 established procedures. These programs were designed to facilitate the development and
271 expedite the review of new drugs intended to treat serious or life-threatening conditions and that
272 demonstrate the potential to address unmet medical needs. The Best Pharmaceuticals for
273 Children Act (BPCA)[25] and the Pediatric Research Equity Act of 2003 (PREA)[26] may also apply
274 to drugs developed under the Animal Rule.
275
276 Sponsors should note that FDA may seek input from advisory committees for various issues
277 related to the Animal Rule. Issues for discussion can include whether the Animal Rule is the
278 appropriate regulatory development pathway for drugs intended for a specific indication,
279 concurrence on the natural history model of a disease or condition, the acceptability of the use of
280 an animal model with a specific investigational drug, the design of adequate and well-controlled
281 animal efficacy studies, and the adequacy of data to support approval. In some instances, more

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[21] Section 505(b)(5)(B) of the FD&C Act (as amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013, Public Law 113-5) provides for the use of special protocol assessment provisions "in the case where human efficacy studies are not ethical or feasible, of animal and any associated clinical trials which, in combination, are intended to form the primary basis of an effectiveness claim."

[22] For procedural information, see FDA's guidance for industry *Special Protocol Assessment*.

[23] FDA has issued a draft guidance on this topic. When the guidance on *Expedited Programs for Serious Conditions–Drugs and Biologics* is finalized, it will represent the Agency's current thinking on the topic.

[24] For information on the Orphan Drug Designation program, see the following Web page at http://www.fda.gov/ForIndustry/DevelopingProductsforRareDiseasesConditions/HowtoapplyforOrphanProductDesignation/ucm135122.htm.

[25] See Public Law 107-109.

[26] See Public Law 108-155.

282 than one advisory committee meeting may be warranted at different time points in a single
283 development program.
284
285 **B.   Access to Investigational Drugs During a Public Health Emergency**
286
287 Data collected from animal efficacy studies may support the emergency use of drugs under an
288 investigational new drug (IND) application or an emergency use authorization (EUA).[27] FDA's
289 decision to allow emergency use of a drug under an IND or EUA will be made on a case-by-case
290 basis, taking into account the anticipated or actual emergency, size of the affected population,
291 data included in the submission, and risk-benefit analysis. Neither a decision to allow
292 emergency use of the drug under an IND or EUA nor data submitted in support of either
293 mechanism should be viewed as a final drug development goal. FDA emphasizes that drug
294 development and systematic data collection should continue to obtain the body of evidence to
295 support drug approval under the Animal Rule and associated postmarketing requirements.
296
297 **C.   Communications With FDA**
298
299 Sponsors are encouraged to hold discussions with FDA in the early stages of a drug development
300 program. Sponsors unsure of the appropriate regulatory review division or office for their
301 investigational drugs can inquire through the electronic mailbox, CDER-CBER-
302 ARJurisdiction@fda.hhs.gov, provided by FDA's Center for Drug Evaluation and Research
303 (CDER) and Center for Biologics Evaluation and Research (CBER) for this sole purpose.
304
305 Sponsors should consult Agency guidance regarding the process and expectations for formal
306 meetings.[28] Early in the drug development process, the sponsor and the review division should
307 discuss the avenues and expectations for communication for addressing extenuating or
308 unforeseen circumstances. It is the sponsor's responsibility to build sufficient time into the
309 development plan to permit the review, discussion, and resolution of issues prior to the initiation
310 of relevant studies. FDA will try to accommodate the sponsor should unforeseen circumstances
311 arise.
312

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[27] Expanded access for individual patients (including for emergency use), intermediate-size patient populations, and large patient populations (under a treatment IND or treatment protocol) are described in 21 CFR 312.300-320. FDA has issued a draft guidance on this topic. When the guidance on *Expanded Access to Investigational Drugs for Treatment Use – Qs & As* is finalized, it will represent the Agency's current thinking on this topic.

EUA criteria are described in FDA's guidance *Emergency Use Authorization of Medical Products.* Individual patient INDs are not a feasible strategy for large-scale events requiring mass access to an investigational drug. Sponsors anticipating multiple access requests for an investigational drug should discuss proposals for IND protocols with FDA.

[28] See FDA's guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants*.

313 Some of the drug development issues that should be the subject of meetings with FDA[29] will
314 differ from those for drugs developed under other regulatory pathways. Examples of issues for
315 Animal Rule drug development discussions are listed in section III.A.

316

317 **D.   Animal Model Qualification Program**

318

319 The Animal Model Qualification Program (AMQP)[30] is jointly supported by CDER and CBER
320 to address the need for publicly available animal models for use in drug development under the
321 Animal Rule.[31] Through this program, animal models are evaluated and qualified for a specific
322 context of use (COU) that describes the appropriate use and application of the qualified animal
323 model in drug development and regulatory review and specifies the details[32] necessary to
324 replicate the model. Submitting a model for qualification is voluntary. Approval under the
325 Animal Rule does not require the use of a qualified model.

326

327 Qualification is a regulatory conclusion[33,34] that is not linked to a specific drug. Qualification of
328 an animal model through the AMQP indicates that FDA has accepted that a specific animal
329 species, given a specific challenge agent by a specific route, produces a disease process or
330 condition that in multiple important aspects corresponds to the human disease or condition of
331 interest. Once the animal model is qualified, FDA does not have to reevaluate this conclusion
332 each time this qualified model is used within the bounds of its stated COU.

333

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[29] Section 565(d) of the FD&C Act (as amended by the Pandemic and All-Hazards Preparedness Reauthorization Act of 2013, Public Law 113-5) provides that sponsors developing countermeasures under the Animal Rule may request and receive two meetings with FDA, one to discuss "proposed animal model development activities" and the other "prior to initiating pivotal animal studies." These meetings and procedures for obtaining such meetings are within the scope of FDA's guidance for industry *Formal Meetings Between the FDA and Sponsors or Applicants* and satisfy this requirement.

[30] The AMQP was established under FDA's Drug Development Tools (DDT) Qualification Programs. Additional information about qualifying animal models can be accessed through the Animal Model Qualification Program Web page at
http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm284078.htm.

[31] Qualification of an animal model is voluntary and is limited to animal models developed for the intended purpose of supporting the development programs for multiple investigational drugs for the same targeted disease or condition. A model developed by a sponsor of an investigational drug for the intended purpose of use in the development program of that drug alone will not be eligible for qualification.

[32] These details include, but are not limited to, the following: characterization of the animals to be used, characterization and preparation of the challenge agent, procedural information for the challenge agent exposure, identification of the primary and any secondary efficacy endpoints, triggers for intervention, and ranges of values of key parameters of the disease or condition that will be used as measures of quality control and quality assurance when the model is replicated.

[33] Woodcock, J, S Buckman, F Goodsaid, MK Walton, I Zineh, 2011, Qualifying Biomarkers for Use in Drug Development: A US Food and Drug Administration Overview, *Expert Opin Med Diagn*, 5(5):369-374.

[34] The qualification recommendation for the animal model and its COU will be made publicly available and can be referenced by its FDA-assigned tracking number for use in regulatory submissions.

334 Before using a qualified animal model of a disease or condition in an adequate and well-
335 controlled efficacy study, the sponsor of an investigational drug should establish that the model
336 is a suitable test system for the drug with regard to the drug's mechanism of action and related
337 host factors and the ability to select a dose and regimen in humans (see section V.B). Similarly,
338 since animal models are qualified without reference to a specific drug, the use of the qualified
339 animal model does not ensure that the model will be found acceptable as "a single animal species
340 that represents a sufficiently well-characterized animal model for predicting the response in
341 humans" as stated in the second criterion for drug approval under the Animal Rule. FDA may
342 not accept evidence of effectiveness from a single animal model (even if it is qualified) for an
343 investigational drug, unless FDA concludes there is sufficient evidence that the results generated
344 in this model adequately predict the response to the drug in humans. The regulatory decision to
345 allow approval of a drug based on the use of an animal model in a single species will be made by
346 the review division on a case-by-case basis (see section II).

348 Since qualification is a regulatory conclusion, FDA recommends the use of GLP, to the extent
349 practicable, for the model-defining natural history studies[35] submitted to support the qualification
350 of an animal model, to facilitate study conduct in a manner that ensures data quality and
351 integrity. The model-defining natural history studies submitted for qualification will be subject
352 to inspection by FDA to verify the quality and integrity of the data (see section IV.B).

355 **IV.   ANIMAL STUDIES – GENERAL EXPECTATIONS**

357 The discussions in this section are focused on the Animal Rule-specific studies, i.e., the natural
358 history studies that define the animal model in which efficacy will be tested, the adequate and
359 well-controlled animal efficacy studies, and the pharmacokinetic (PK) and/or pharmacodynamic
360 (PD) studies in animals used to select a dose and regimen in humans.

362   **A.   Animals Used in Investigations**

364 For the Animal Rule-specific studies, the number of animals should be determined to ensure
365 scientifically valid results. Well-designed experiments use a sufficient number of animals to
366 achieve the scientific objective, include the necessary control groups, and incorporate
367 appropriate statistical analyses.

369 Animal Rule-specific studies typically include a small number of animals. To aid in the
370 interpretation of these studies, the variability among animals should be minimized within each
371 study and across the related studies. Appropriately designed protocols generally control for age,
372 body weight, current health status, and the physical environment of the test animals. For rodents,
373 it is possible to control for genetic variability, prior nutrition, and previous exposure to
374 pathogens, although this is generally not possible for non-rodent species such as rabbits, dogs,

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[35] In the context of animal model qualification, the *model-defining natural history studies* are the animal studies that establish the ranges of values of key parameters of the disease or condition that will be specified in the COU for the qualified model and that will be used as measures of quality control and quality assurance when the model is replicated.

375 and nonhuman primates. The animals should be research naïve. Any prior research experience,
376 even as a control animal, has the potential to cause stress and alter an animal's physiological
377 responses.
378
379 Appropriate inclusion and exclusion criteria for the acceptance of the animals into the study
380 should be pre-specified and discussed with FDA before initiating the studies. The information
381 that should be provided for the characterization of individual animals used in the investigation is
382 described in section IV.D.
383
384   **B.   Study Conduct**
385
386 The adequate and well-controlled animal efficacy studies and the PK and/or PD studies in
387 animals used to select a dose and regimen in humans should be conducted in a manner that
388 ensures data quality and integrity, as would be expected for studies submitted to establish
389 effectiveness and support the labeling of a drug approved under a traditional regulatory pathway.
390 There are no regulations that specifically address data quality and integrity issues for Animal
391 Rule-specific studies. The Good Laboratory Practice for Nonclinical Laboratory Studies
392 regulations[36] (GLP) were developed as a quality system for nonclinical safety studies.
393 Nonetheless, GLP provide a framework (e.g., definitions, procedures, roles and responsibilities,
394 and controls) for the conduct of nonclinical studies, and FDA considers GLP to be a well-
395 established and relevant system for ensuring data quality and integrity for the adequate and well-
396 controlled animal efficacy studies and the PK and/or PD studies in animals used to select a dose
397 and regimen in humans. FDA, therefore, recommends the use of GLP for these studies[37] to the
398 extent practicable.
399
400 There may be justifiable limitations in the ability to apply GLP when conducting these studies,
401 especially for those using challenge agents that require high containment facilities. Before
402 initiating these studies, sponsors should identify aspects of the studies anticipated to be a
403 challenge with regard to GLP and propose methods for adapting the studies to ensure the quality
404 and integrity of the resulting data. Sponsors should seek concurrence from FDA on the data
405 quality and integrity plan before the studies are initiated.
406
407 The adequate and well-controlled animal efficacy studies and the PK and/or PD studies in
408 animals used to select a dose and regimen in humans serve as the basis for a regulatory action
409 (e.g., approval) under the Animal Rule. Thus, FDA has the authority to inspect these studies
410 prior to taking an action. Inspections will be conducted to verify the quality and integrity of the
411 raw data, supporting documentation, facilities, equipment, and the results submitted to FDA in
412 the final report. *Quality* includes, but is not limited to, whether the study was conducted in
413 accordance with the protocol, standard operating procedures, and applicable standards of
414 research. *Integrity* includes, but is not limited to, the assurance that the raw data and

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[36] See 21 CFR 58.

[37] In addition, FDA recommends the use of GLP, to the extent practicable, for the *model-defining natural history studies* submitted to support the qualification of an animal model (see section III.D). Qualification is a regulatory conclusion, and thus, these studies should be conducted in a manner that ensures data quality and integrity.

415 documentation are consistent with reported results. FDA will verify that study personnel
416 followed the agreed upon data quality and integrity plan. Inspectional observations will be
417 shared with the inspected entity and evaluated by the review division to determine the impact of
418 the observations on the acceptability of the data to support drug approval.
419
420 Animal studies conducted in the United States and its territories must comply with applicable
421 laws and regulations as prescribed by the Animal Welfare Act[38] and the Public Health Service
422 Policy on Humane Care and Use of Laboratory Animals.[39] All studies should comply with
423 general principles for the care and use of animals in biomedical research (see Appendix A).
424 Sponsors should ensure that adequate safety and security provisions are in place for all studies
425 when needed. For example, for select agents and toxins, sponsors must adhere to the regulations
426 found under 42 CFR part 73 and, when applicable, sponsors should comply with standards on the
427 use of biosafety level (BSL) laboratory facilities.[40]
428
429 The investigational drug used in the adequate and well-controlled animal efficacy studies and the
430 animal PK and/or PD studies used to select a dose and regimen in humans ideally should be
431 manufactured under current good manufacturing practice regulations.[41] The investigational drug
432 also should be as close as practicable to the to-be-marketed drug; any differences should be
433 discussed with the review division before studies are initiated.
434
435   **C.**   **Types of Animal Care Interventions**
436
437 As used in this guidance, animal care interventions in animal studies are divided into three
438 categories based on the rationale for their use: (1) intervention as part of adequate veterinary
439 care, (2) intervention to permit the manifestation of the disease or condition of interest for the
440 purpose of model development, and (3) intervention as supportive care to mimic the human
441 clinical scenario. These categories of interventions are discussed individually in Appendix B.
442 The potential effects of the interventions on the animal (e.g., toxicity, effects on the immune
443 system) and on the PK, PD, and efficacy of the investigational drug should be considered in the
444 design and interpretation of each study. In addition, protocols for the adequate and well-
445 controlled efficacy studies should include plans for addressing the impact of potential differences
446 in care among animals.
447

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[38] See 7 U.S.C. 2131 et seq.

[39] National Institutes of Health, Office of Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," 2002, http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on November 21, 2013.

[40] U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health, 2010, *Biosafety in Microbiological and Biomedical Laboratories,* 5th Edition, Atlanta, GA: CDC.

[41] See 21 CFR 210 and 21 CFR 211.

448   **D.**   **The Study Report**
449
450   FDA expects that complete, final study reports will be submitted for the Animal Rule-specific
451   studies. Complete study reports should include, but are not limited to, the following:

452   •   The prospectively designed protocol, including all protocol amendments, the
453   prospectively designed statistical plan, and a description of all protocol deviations

454   •   Detailed descriptions of the elements of the study design, including the characterization
455   of the animals used in the study;[42] information on the formulations and administration of
456   the investigational drug and controls; and information on the characterization,
457   preparation, and delivery of the challenge agent

458   •   A comprehensive description of study procedures

459   •   The results[43] of each parameter or variable evaluated at each time point in the study and
460   any unscheduled medical intervention

461   •   The final audited study report that includes analyses and interpretation of the study data
462   and explanation of any deviations from the agreed upon plan for data quality and integrity
463
464   Preliminary plans for collection, organization, format, and level of detail of study data should be
465   discussed with the review division before conducting these studies. Sponsors are encouraged to
466   submit prototype versions of the study datasets prior to finalization of datasets.

467
468   **E.**   **Submission of the Study Report and Data**
469
470   FDA strongly encourages the submission of study data in a standardized electronic format to
471   support analysis and review. Sponsors should consider the implementation of data standards and
472   seek FDA feedback as early as possible in the animal model and drug development lifecycle, so
473   that the data standards are included in the design, conduct, and analysis of studies.[44]
474

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[42] The individual animal information should include, when appropriate, species, strains and substrains (when applicable), breed (when applicable), age, gender, body weight, vendor source, origin of the animal (to the extent known), procedures for identification and individual animal identification, physiological status (e.g., adult, juvenile, lactating, and pregnant), data collected during routine husbandry prior to protocol assignment, including pre-study health screen, health records, medications or therapies administered pre- and post-protocol assignment, and an adequate description of housing and husbandry conditions. For individual animal tracking purposes, a table that cross-references the unique animal identification number for the study, treatment allocation, fate or disposition, and chain of custody should be submitted. For each animal assigned more than one identification number during life, the table also should include reference to all other identification numbers (e.g., a unique animal number assigned by the source).

[43] These results should include group summary tabulations, line listings of the results for each individual animal, copies of the individual animal case report forms (all veterinary medical records), and any other primary data necessary for the reconstruction of key analyses and evaluation of the study report.

[44] Information is available through FDA's Web page, Study Data Standards Resources, available at http://wcms.fda.gov/FDAgov/ForIndustry/DataStandards/StudyDataStandards/default.htm.

475 The Electronic Common Technical Document (eCTD) is the standard format for regulatory
476 submissions to CBER and CDER. The eCTD does not provide a specific location for the natural
477 history or model characterization studies conducted in animals and for the adequate and well-
478 controlled animal efficacy studies. Their locations within electronic submissions have varied.
479 For consistency, it is recommended that these studies be submitted to Module 4 (Nonclinical
480 Study Reports), section 4.2.1.1 (Primary Pharmacodynamics). This recommendation does not
481 determine the disciplines of the primary reviewers for the studies; that decision is the purview of
482 the FDA review division.
483
484
485 **V.   ESSENTIAL ELEMENTS OF AN ANIMAL MODEL**
486
487 The selection of an animal model for an efficacy study should be based on its adequacy as a
488 model of the human disease or condition and its suitability with regard to the investigational
489 drug. Section V.A describes the elements related to the disease or condition induced by the
490 etiologic or challenge agent.[45] It is the sponsor's responsibility to provide, to the fullest extent
491 possible, a documented summary of the etiologic agent-induced human disease or condition and
492 a detailed discussion that delineates how these data support selection of the proposed animal
493 model. Evidence supporting the relevance of an animal model to a human disease or condition
494 can be obtained from various sources[46] that provide adequate documentation of study quality.[47]
495 For example, data from literature or historical studies may support the use of an animal model
496 when the reports include a level of detail that is sufficient to assess the appropriateness of the
497 animal model. The source, organization, format, and level of detail of the available study data
498 should be discussed with the review division before submitting the data.
499
500 Section V.B describes elements related to the investigational drug and the selection of an
501 effective dose in humans. The sponsor should provide a justification of the suitability of each
502 model based on the investigational drug's mechanism of action, dosage form, and route of
503 administration, and the method proposed for selection of a dose in humans. Issues related to
504 animal model development for one or more investigational drugs that are to be developed for use
505 in combination or concurrently are beyond the scope of this guidance and should be discussed
506 with the review division.
507
508 The following essential elements should be considered in the development and/or the selection of
509 an animal model.[48] Any element not achievable for an etiologic or challenge agent or drug under
510 investigation should be discussed with FDA.
511

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[45] As used in this guidance, the term *etiologic agent* refers to the substance causing the disease or condition in humans. The term *challenge agent* refers to the substance used to cause the disease or condition in the animal studies.

[46] Comparable to the sources of clinical data described in 21 CFR 314.50(d)(5)(iv).

[47] Comparable to the discussion of the documentation of the quality of evidence described in FDA's guidance for industry *Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products.*

[48] See section IX for the associated Checklist of Essential Elements of an Animal Model.

512   **A.**   **Elements Related to the Etiologic or Challenge Agent-Induced Disease or**
513   **Condition**
514
515   *1.*   *Characteristics of the Etiologic or Challenge Agent That Influence the Disease or*
516   *Condition*
517
518   The characteristics of the specific etiologic or challenge agent that influence the disease
519   or condition under study include its pathophysiological mechanisms of toxicity or
520   virulence, the route of exposure, and the dose and quantification of exposure. These
521   characteristics are discussed below.
522
523   a.   The Challenge Agent
524
525   The challenge agent used to establish the disease or condition in the animal
526   studies generally should be the same as the etiologic agent that causes the human
527   disease or condition. If the challenge agent is different from the etiologic agent
528   known to cause the human disease or condition, the sponsor should provide
529   justification for the use of that challenge agent. The sponsor also should explain
530   why, when used in the proposed animal model, the challenge agent should be
531   considered suitable for establishing effectiveness of the investigational drug in
532   humans against the intended etiologic agent. For example, for an animal efficacy
533   study to support approval of a drug to treat the gastrointestinal subsyndrome of
534   acute radiation syndrome (GI-ARS), a sponsor may not be able to predict the
535   actual radiation exposure that would follow a nuclear detonation or the
536   subsequent fallout. In such a case, the sponsor should provide a detailed
537   explanation of the appropriateness of the type and dose of radiation used in the
538   study and their relevance to the clinical situation.
539
540   The selection of a biological challenge agent should be based on known virulence
541   factors, using standardized, validated test methods, and the challenge agent used
542   ideally should be of low passage history. For plague studies conducted in
543   animals, pigmented *Y. pestis* strains are preferred, as non-pigmented strains rarely
544   cause disease. Generally, bacterial and viral strains known to be associated with
545   outbreaks of human disease should be used for the natural history and animal
546   efficacy studies (e.g., Ebola Zaire virus isolated from a human who died from an
547   infection during an outbreak should be used in the animal studies); however, there
548   may be issues regarding differences in the strain or serotype of the biological
549   agent that will limit the relevance, or preclude the use, of data obtained to support
550   the proposed clinical indication. For example, there may be various strains of a
551   bacterium that differ in the expression of virulence factors. When an
552   investigational drug targets a particular virulence factor or pathogenic mechanism
553   associated with a particular virulence factor, effectiveness may be limited to
554   strains that express that particular virulence factor, and an indication for all
555   variants of that bacterium may not be possible.
556

557   The challenge agents and their preparations should be characterized in terms
558   relevant for their category (i.e., biological, chemical, radiological, or nuclear).
559   For biological agents, these terms should include passage history, method of
560   preparation, concentration, and number of organisms per dose. For chemical
561   agents, characteristics should include source and stated purity of the agent, dosing
562   formulation, concentration, and stability under the conditions of use. For
563   radiation or nuclear challenges, the terms should include the type and source of
564   radiation. Such characterization facilitates comparison among studies.
565
566   b.   Pathophysiological Mechanisms of Toxicity or Virulence
567
568   The pathophysiological mechanisms of toxicity or virulence of the challenge
569   agent expressed in the animal model should be similar to those expressed by the
570   etiologic agent in humans. For a biological agent, the pathophysiological
571   mechanisms of virulence are the pathogenic determinants of the microbe (i.e., its
572   genetic, biochemical, or structural features that enable it to elicit disease in a
573   host). Examples of microbial pathogenic determinants include toxins, substances
574   that promote invasion, substances that modulate inflammation, substances that
575   cross-react with host tissues, and mechanisms to evade host defenses. For a
576   chemical agent, the mechanisms of toxicity can include receptor binding,
577   inhibition of enzymes, and binding of intracellular components. For radiation, the
578   mechanisms of toxicity include DNA damage and the generation of free radicals.
579
580   c.   Route of Exposure
581
582   When the pathogenesis of the disease or condition is dependent on the route of
583   exposure to the challenge agent, the animal models should use the same route as
584   that anticipated in humans. For example, human infection with *Y. pestis* can
585   occur through flea bite or inhalational exposure. Exposure through a flea bite
586   usually leads to development of bubonic plague, whereas inhalational exposure
587   leads to the development of pneumonic and septicemic plague. Thus, an animal
588   model of pneumonic plague should use an inhalational route of exposure to *Y.*
589   *pestis*.
590
591   In cases when the challenge agent-induced disease or condition is not clearly tied
592   to its route of exposure, alternate routes of exposure may be acceptable. If a
593   sponsor proposes to use a route of exposure to the challenge agent in animals that
594   is different from that expected in humans, scientific justification should be
595   provided. If such an approach is under consideration, it should be discussed with
596   FDA before initiation of the natural history and animal efficacy studies.
597
598   Sponsors should discuss potential paths forward with FDA when trying to
599   develop a drug for a disease or condition for which limited or no human data are
600   available for the etiologic agent by the route of exposure in the proposed clinical
601   indication.
602

603   d.   Dose and Quantification of Exposure
604
605   Ideally, the sponsor should use a challenge agent dose that produces a disease or
606   condition in animals that corresponds to the expected extent and severity of the
607   human disease or condition. The dose of the etiologic agent that causes the
608   human disease or condition may not be known, or the exposure cannot be fully
609   quantified. For example, following a nuclear incident, the radiation exposure to
610   humans may not be readily quantifiable. In such a case, a sponsor developing a
611   drug to treat the hematopoietic subsyndrome of acute radiation syndrome (H-
612   ARS) should provide a detailed description of the methods of radiation exposure
613   used in the animal studies, including type and source of radiation, dose and dose
614   rate, whole versus partial body irradiation, and their relevance to the clinical
615   situation.
616
617   The method for the delivery of the challenge agent should be described in
618   sufficient detail to permit replication of test conditions. Reliable quantification
619   using a validated assay and reproducibility of the challenge agent dose should be
620   demonstrated from model development through its use in the animal efficacy
621   studies. In general, the target dose and actual dose delivered to an individual
622   animal should be expressed in absolute terms (e.g., colony forming units or
623   plaque forming units for a biological agent, or the radiation dose expressed in
624   gray) as well as in terms that indicate the toxicity or virulence of the challenge
625   agent (e.g., the LD_{50}, which is the dose sufficient to kill 50% of those exposed to
626   the agent).
627
628   2.   *Host Susceptibility and Response*
629
630   The animal species chosen for model development should be susceptible to the challenge
631   agent. Also, if the host immune response is part of the pathogenesis of the disease or
632   condition in humans, it should play a similar role in the animal model. FDA recognizes
633   there may be susceptibility differences among species. For example, an animal species
634   used to study the efficacy of a treatment for H-ARS may require a different threshold of
635   radiation exposure to develop the subsyndrome than the threshold that is needed in
636   humans. If the thresholds in humans and in the animal model differ greatly, the
637   suitability of the animal model may be called into question and the model should be
638   discussed with FDA. The animal species may still be appropriate for study if the
639   resulting disease or condition and time course of progression are similar in the animal
640   species and humans. The factors that determine differences in susceptibility to the agent
641   should be described to the extent possible. For example, when selecting an animal model
642   to study the lethal effects of soman, an important consideration is the endogenous level of
643   carboxylesterase in the selected animal species. Certain animal species are less

644   susceptible to the effects of soman, because the carboxylesterase enzyme has a
645   detoxifying effect on soman.[49]
646
647   Animal species that are not susceptible to the etiologic agent may not be suitable models
648   for efficacy studies. Other approaches to the accrual of relevant animal data may need to
649   be explored (for an example, see the discussion of the variola virus and human smallpox
650   in section II).
651
652   The response to the challenge agent (i.e., the resulting disease or condition) manifested
653   by the animal species should be similar to the disease or condition seen in humans
654   exposed to the etiologic agent with respect to the proposed clinical indication. For
655   example, mustard gas typically produces extensive blistering to exposed human skin. If
656   the animal species evaluated does not have blistering as a prominent feature of exposure
657   to mustard gas, it is unlikely that this animal model will be acceptable to FDA for the
658   development of a treatment for mustard gas-induced injury to the skin. Similarly, mice
659   are known to be susceptible to *Bacillus anthracis*; however, the pathogenesis of the
660   disease process in mice differs from that in humans. Therefore, mice may not be
661   appropriate models for anthrax efficacy studies.[50] If the sponsor believes that such a
662   model is supportive to the study of their investigational drug, a justification should be
663   provided and the model should be discussed with FDA before proceeding.
664
665   *3.*   *Natural History of the Disease or Condition – Pathophysiological Comparability*
666
667   The general expectations for the design and conduct of animal natural history studies are
668   described in Appendix C. The natural history of the disease or condition in the selected
669   animal species and in humans should be characterized and the similarities and differences
670   compared and contrasted. This information should be discussed with FDA before
671   initiation of the efficacy studies. To facilitate these discussions, sponsors should provide
672   an adequately documented summary of the etiologic agent-induced human disease or
673   condition and a detailed discussion as to how these data support the selection of the
674   animal model. This information should include (but not be limited to) the following
675   parameters:

676   •   Time from exposure to onset of the manifestations of disease or injury

677   •   Time course of the progression

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[49] Pretreatment with pyridostigmine bromide was shown to decrease the lethality of soman in rhesus macaques and guinea pigs. Pyridostigmine bromide's protective effect was not consistently demonstrated in other species tested because these other species were protected from soman by high levels of endogenous carboxylesterase, an enzyme that detoxifies soman. To confirm the theory for inter-species differences, a study was conducted in rats pretreated with a carboxylesterase inhibitor before exposure to soman. Rats pretreated with pyridostigmine bromide demonstrated decreased lethality, compared to rats not pretreated with pyridostigmine bromide. These results were similar to the survival benefit demonstrated with pyridostigmine bromide in the rhesus macaques.

[50] Leffel, EK and MLM Pitt, "Characterization of New and Advancement of Existing Animal Models of *Bacillus anthracis* Infection," in JR Swearengen (ed.), *Biodefense Research Methodology and Animal Models*, Boca Raton, FL: CRC Press, 2012, pp. 81-98.

678   • Manifestations (e.g., signs and symptoms, clinical and pathological features,
679   laboratory parameters, extent of organ involvement, morbidity, and outcome)
680
681   These parameters can be influenced by many factors, such as the type of etiologic or
682   challenge agent, virulence or lethal potential of the etiologic or challenge agent, route of
683   exposure, concentration, host factors including immune status, and medical management
684   in humans versus animal care interventions. Potential endpoints for evaluating efficacy
685   also should be discussed. Experimental parameters may need to be modified to create a
686   disease or condition that more closely mimics that seen in humans, or the model may
687   need to be tailored for the proposed clinical indication.
688
689   It may not always be possible to compare the pathophysiology of the disease or condition
690   in animal models to that in humans. For some diseases or conditions, relevant human
691   data are not available, or the data are limited to references in the literature describing the
692   end-stage pathology for symptomatic patients. For example, the description of the
693   pathophysiology of H-ARS has been derived mainly from the literature discussing
694   accidental occurrences in which humans received variable exposures to radiation.
695
696   a.   Time to Onset
697
698   The time to onset of the disease or condition in animals should be reasonably
699   similar to that in humans. Factors such as route of exposure, level of exposure
700   (e.g., dose, concentration), and species or strain of the infective microorganism
701   can influence time to onset and should be taken into consideration in model
702   development.
703
704   b.   Time Course of Progression
705
706   Ideally, the progression of the disease or condition in the selected animal models
707   should be similar to that seen in humans; when different, it should allow time for
708   identification of the disease or condition, intervention, and assessment of the
709   outcome of treatment. Demonstration of the effect of the investigational drug
710   may be more challenging when the time between onset and death is short. For
711   example, hamsters challenged with *B. anthracis* have such a rapid disease
712   progression that this species is not useful for testing the efficacy of drugs for the
713   treatment of anthrax in humans. The route of exposure may affect the progression
714   of the disease or condition, including the time course.
715
716   c.   Manifestations
717
718   The manifestations of the disease or condition, including laboratory parameters,
719   histopathology, gross pathology, and outcome (morbidity and/or mortality), and
720   their known time course should be compared between untreated animals and
721   humans (e.g., historical information from human cases). Differences should be
722   clearly noted and explained based on the understanding of the pathophysiological
723   differences between the species, when possible. Certain manifestations in humans

724   (e.g., fever, shortness of breath) may be difficult to discern in animals through
725   clinical observation; therefore, a sponsor may need to use more refined
726   techniques, such as telemetry, to evaluate affected animals. Animals in the
727   natural history studies and the efficacy studies should be observed with greater
728   frequency over the entire course of the day than would be typical of most animal
729   studies used for toxicology evaluation. The frequency of observations per day
730   may vary over the course of the study, depending on the animal species and strain,
731   the experimental conditions, and the mechanism of disease or injury of the
732   challenge agent. The observation frequency should be adequate to characterize
733   the course of disease or condition and to define the desired treatment triggers and
734   efficacy endpoints.
735
736   When the primary endpoint is mortality, animals should be evaluated in the
737   context of prospectively defined euthanasia criteria. With a mortality endpoint,
738   animal welfare and sample integrity should be addressed. Sample integrity may be
739   compromised if not obtained prior to or immediately after death or euthanasia.
740   Study results may be influenced by the euthanasia criteria used. Study personnel
741   should be blinded to exposure and/or treatment status and should follow the
742   observation frequency paradigm and euthanasia criteria to minimize the
743   possibility of unnecessary suffering of moribund animals and to reduce potential
744   study bias as much as possible.
745
746   *4.*   *Trigger for Intervention*
747
748 A clearly defined trigger for intervention should be established for use in animal efficacy
749 studies when needed (e.g., post-exposure prophylaxis and treatment indications). The
750 trigger for intervention should be identified based on the natural history studies. For a
751 post-exposure prophylaxis indication, a trigger for intervention should be defined to
752 ensure drug administration within a reasonable timeframe after exposure to the challenge
753 agent and prior to the onset of the disease or condition of interest. The timeframe should
754 be justified with respect to administration of the drug to humans. Animals cannot
755 simulate the health-seeking behavior manifested by humans; therefore, a clearly defined
756 trigger for intervention for a treatment indication will ensure that treatment is not initiated
757 until the disease or injury process is established. If signs observed in the animal model
758 closely resemble those in humans and are predictive for the disease, they may serve as the
759 trigger for intervention.
760
761 In the absence of disease- or condition-defining manifestations, sponsors can propose a
762 biomarker as a trigger for intervention, if information can be provided that it correlates to
763 the pathophysiology of the disease or condition. The utility of the biomarker should be
764 justified through an analysis that correlates the time course of the appearance of the
765 parameter in animals with the onset of the disease or condition in the animals. The assay
766 method and its performance characteristics for a biomarker used as a trigger for
767 intervention in animal studies should be adequately described.
768

769   Sponsors are encouraged to initiate early discussions with FDA regarding the utility of
770   the chosen triggers for intervention, particularly when the manifestations of the disease or
771   condition in the animals differ from those in humans, or when a biomarker is used as a
772   trigger for intervention.
773
774   **B.**   **Elements Related to the Investigational Drug and the Selection of an**
775   **Effective Dose in Humans**
776
777 The concepts discussed in this section apply primarily to drugs and therapeutic proteins. For
778 information regarding preventive vaccines and cellular and gene therapies, consult sections
779 VII.A and VII.B, respectively.
780
781   *1.*   *The Investigational Drug*
782
783   The characterization of the investigational drug with regard to identity, concentration,
784   purity, composition, and stability is the same under the Animal Rule as for any
785   investigational drug developed under other regulatory pathways. Additional elements of
786   the investigational drug that are important considerations for animal model selection
787   include the mechanism of action, drug class, dosage form, and route of administration.
788   These elements are discussed below.
789
790   a.   Mechanism of Action
791
792   Approval under the Animal Rule requires a reasonable understanding of the
793   investigational drug's mechanism of action with regard to its ability to prevent or
794   substantially reduce the toxic effects of the challenge agent.[51] The sponsor should
795   relate the mechanism of action of the drug in the proposed animal species to the
796   presumed mechanism of action in the human. This information is critical to the
797   selection of appropriate animal species in which to test the efficacy of the
798   investigational drug and the interpretation of the results of those studies. The
799   drug's effect in the animal species is expected to be predictive of the drug's effect
800   in humans.[52]
801
802   An understanding of the mechanism of action of the investigational drug may help
803   in the identification of specific safety or efficacy issues, the interpretation of
804   findings in the proposed animal studies, and the identification of additional
805   studies that should be performed. This understanding also may lead to the
806   identification of a relevant biomarker for potential use in selecting a dose and
807   regimen in humans (see section V.B.2.b. for further discussion).
808

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[51] See 21 CFR 314.610 (a)(1) for drugs; 21 CFR 601.91 (a)(1) for biological products.

[52] See 21 CFR 314.610(a)(2) for drugs; 21 CFR 601.91(a)(2) for biological products.

809   b.   Drug Class

810

811   Information that is available about other drugs that are members of the same

812   therapeutic class or pharmacologic class as the investigational drug can be used to

813   help identify potential animal models. This information also may help anticipate

814   safety and efficacy issues in the proposed animal model and in the projected

815   human use.

816

817   c.   Dosage Form and Route of Administration

818

819   The suitability of the dosage form and route of administration with regard to the

820   proposed indication should be considered in the development of the drug. For

821   example, an oral dosage form may be preferred for post-exposure prophylaxis for

822   large populations while an intravenous dosage form may be more appropriate for

823   seriously ill patients.

824

825   To the extent practicable, drug administration in the animal and human studies

826   should be comparable to the expected clinical use of the investigational drug (e.g.,

827   dosage form, route of administration, to-be-marketed formulation). Comparative

828   bioavailability information may be necessary to bridge PK across studies, for

829   example, when changes in formulation occur during development. If multiple

830   dosage forms or routes of administration are being developed, sponsors should

831   discuss with the review division the types of PK data that may be needed to

832   support the approval of each.

833

834   2.   *Selection of an Effective Dose in Humans*

835

836 The Animal Rule requires that PK and PD data or information (or other relevant data or

837 information) for the investigational drug[53] be sufficient to permit the selection of a dose

838 and regimen expected to be effective in humans.[54] The methods used for selecting an

839 effective human dose may differ based on factors including, but not limited to, the target

840 of the investigational drug, prior human experience in related indications, and the

841 availability of a relevant biomarker. Several approaches to the selection of an effective

842 dose for humans are described in section V.B.2.b.

843

844   Agency concurrence on the animal model in which the efficacy of an investigational drug

845   will be tested will be contingent, in part, on the ability to select an effective dose and

846   regimen in humans. Sponsors are encouraged to initiate discussions with FDA on the

847   proposed rationale for human dose selection early in their drug development program.

848   Protocols for animal PK and efficacy studies should include adequate plans for

---

[53] This section focuses on the investigational drug as the active moiety; however, active metabolites also should be considered for the purposes of dose selection. Issues pertaining to active metabolites are handled on a case-by-case basis and should be discussed with the review division.

[54] See 21 CFR 314.610(a)(4) for drugs and 21 CFR 601.91(a)(4) for biological products.

849	assessment of PK and PD data for purposes of defining drug exposure and response
850	characteristics.
851	
852	Issues related to dose selection for the adequate and well-controlled animal efficacy
853	studies for drugs and therapeutic biological products are discussed in section VI.B; for
854	vaccines, see section VII.A.
855	
856	a. PK and PD Information to Be Obtained in Animals and Humans
857	
858	The absorption, distribution, metabolism, and excretion (ADME) of an
859	investigational drug[55,56] should be characterized in animals and humans. In
860	addition, protein binding characteristics and in vitro interaction potential (e.g.,
861	through inhibition, induction, or transporters) should be assessed. As in a
862	traditional drug development paradigm, it is important to ascertain at an early
863	stage of development whether a drug is eliminated primarily by excretion of the
864	unchanged drug or by one or more routes of metabolism.[57] If elimination of the
865	investigational drug is due in part to metabolism, the metabolites should be
866	identified and the metabolizing route(s) should be understood.[58] This information
867	will help identify potential interactions with medical products that are likely to be
868	co-administered based on the clinical scenario and will help predict the
869	consequences of metabolic differences among humans.
870	
871	PK studies should be conducted in healthy animals[59] and healthy human
872	volunteers[60] to characterize the PK profile of the drug in each following the
873	administration of a single dose and multiple doses (if applicable). The assays
874	used for measuring drug concentration in the appropriate body fluids should be
875	validated.[61] As in a traditional drug development program, clinical trials in

---

[55] Biodistribution should be studied for certain products that are not biologically amenable to traditional ADME measures, such as cellular and gene therapies.

[56] Therapeutic biological products do not share the same ADME pathways as small molecules. The ADME characteristics of therapeutic biological products, including receptor-mediated clearance mechanisms leading to nonlinear PK, should be determined.

[57] Sponsors should discuss with the review division whether PK information in specific human subpopulations (i.e., renally and hepatically impaired) also should be obtained.

[58] FDA has issued a draft guidance on this topic. When the guidance on *Drug Interaction Studies – Study Design, Data Analysis, Implications for Dosing, and Labeling Recommendations* is finalized, it will represent the Agency's current thinking on this topic.

[59] The healthy animals used in these studies should be representative of those used in the efficacy studies with regard to key animal characteristics, such as species/subspecies, country of origin, source, age, and weight range.

[60] PK assessments in healthy volunteers may not be possible for some investigational drugs due to the nature of the drug, such as cellular therapies and gene therapies, or due to an unfavorable safety profile of the drug. In such cases, alternative plans should be discussed with the review division.

[61] FDA has issued a draft guidance on this topic. When the guidance on *Bioanalytical Method Validation* is finalized, it will represent the Agency's current thinking on the topic.

876 healthy humans should evaluate safety and PK data over a range of doses. Based
877 on nonclinical and human data, sponsors should discuss the appropriate upper
878 limit for human dosing with the review division, and this agreed upon upper limit
879 should be used to support final human dose selection (see section V.B.2.b for
880 further discussion). The drug exposures associated with efficacy in the adequate
881 and well-controlled animal efficacy studies should be determined. PK
882 information from affected animals[62,63] should be compared to PK information
883 from healthy animals to determine whether the challenge agent-induced disease or
884 condition affects the PK of the investigational drug.

886 The relationships between PK exposure parameters (e.g., area under the plasma
887 concentration-time curve (AUC), peak plasma concentration (Cmax), trough
888 plasma concentration (Cmin), and steady state plasma concentration (Css)) and
889 PD parameters (e.g., efficacy endpoints and potential biomarkers) in the animal
890 models should be determined over a range of at least three doses and the shape of
891 the exposure-response (E/R) curves established in dose range-finding studies. To
892 the extent practicable, protocols for the adequate and well-controlled animal
893 efficacy studies should include adequate plans for PK and PD assessments to
894 enable quantitative E/R analyses.

896 When a biomarker is used as the basis for human dose selection, the assay method
897 and performance characteristics for that biomarker should be adequately
898 described for the animal species and humans.

900 b. PK/PD Considerations for Human Dose Selection

902 PK/PD information can be informative in a number of ways. One approach to the
903 selection of an effective dose for humans takes into account whether the effect of
904 the investigational drug is mediated through its action on the etiologic or
905 challenge agent, rather than on the host (e.g., antimicrobials that target microbial
906 pathogens or investigational drugs intended to bind or detoxify substances such as
907 cyanide or neurotoxins). In such circumstances, it may be possible to use in vitro
908 data (e.g., susceptibility data) to estimate the target concentration/exposure of the
909 investigational drug.[64] The PK/PD parameters that correlate with efficacy should
910 be identified in animal models, and the efficacy of the targeted exposure should
911 be established in adequate and well-controlled animal efficacy studies. The
912 corresponding PK/PD parameters should then be identified in humans. For
913 example, in the case of antimicrobial drugs, in vitro studies can be used to

---

[62] *Affected animals* are defined as those with the challenge agent-induced disease or condition of interest using the animal models proposed for the adequate and well-controlled efficacy studies.

[63] If there are barriers to performing intensive PK sampling in affected animals, sparse sampling approaches can be used.

[64] The extent to which in vitro data may be relevant and useful varies; sponsors should discuss their supporting information with the review division.

914   determine PD characteristics such as susceptibility (e.g., minimum inhibitory
915   concentration (MIC)); then, nonclinical studies can be used to identify potentially
916   relevant PK/PD parameters (e.g., Cmax/MIC ratio, AUC/MIC ratio, the time the
917   concentration remains above the MIC (T>MIC)) that may correlate with an
918   effective response. This information can serve as the basis for the selection of
919   doses to be evaluated in the adequate and well-controlled animal studies to
920   confirm efficacy. Similar PK/PD parameters would then be established for
921   humans to support human dose selection.
922
923   If the investigational drug has been used in humans for other relevant indications,
924   previously established human PK/PD information from those indications may
925   guide dose selection for the animal efficacy studies, which in turn may support
926   selection of the human dose for the proposed indication. For example, existing
927   human E/R data from an antibacterial drug shown to be effective in pneumonia
928   may guide the dose selection for the animal efficacy studies intended to support
929   an indication for the treatment of inhalational plague. Efficacy of the guided dose
930   (e.g., the humanized animal dose) should then be evaluated in the animal model.
931   In some cases, animal studies may suggest that the human dose and regimen
932   needed for the new indication are different from the human dose and regimen
933   used for other indications.
934
935   Another approach for human dose selection may be through the identification and
936   use of an appropriate biomarker. The biomarker should be shown to correlate
937   with the mechanism by which the drug prevents or substantially reduces the
938   etiologic or challenge agent-induced disease or condition and to correlate with the
939   desired clinical outcome (i.e., reduction in mortality or major morbidity). In
940   addition, there should be an ability to determine drug doses for humans that would
941   result in biomarker levels in the desired range based on the biomarker levels
942   associated with efficacy in the adequate and well-controlled animal studies.
943
944   A common and challenging situation is one in which the relationship between the
945   drug exposure and effectiveness is established in animals, but there is no evidence
946   of a relevant link (e.g., biomarker, AUC/MIC) that can predict an effective drug
947   exposure in humans. In this situation, it may be reasonable to assume that the E/R
948   relationship[65] in humans will be similar to the E/R relationship in animals and use
949   a conservative approach to human dose selection (discussed below), based on an
950   understanding of the E/R curve in animals, the exposures associated with a fully
951   effective dose in animals[66] (see Figure 1), and exposures associated with the
952   agreed upon upper limit for human dosing. This approach to human dose
953   selection, based solely on comparing relevant exposure parameters (e.g., AUC,

---

[65] For the purpose of this guidance, the term e*xposure-response relationship* is used broadly to include dose-response relationship.

[66] In most cases, the animal species requiring the highest drug exposure to demonstrate efficacy should be the basis for choosing the human dose.

954   Cmax, Cmin, Css) between humans and animals, should be used only when there
955   is no better alternative.
956
957   **Figure 1**   **A Representative Dose-Response Curve for Survival Based on**
958   **Four Doses of an Investigational Drug Studied in a Well-**
959   **Characterized Animal Model**
960
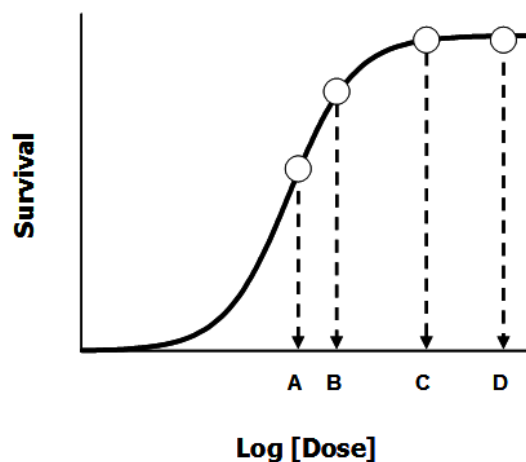
961
962
963   As depicted in Figure 1, survival is increased (compared to placebo) following
964   administration of Doses A, B, C, and D of the investigational drug. The results of
965   the testing of Dose D confirm that Dose C is a fully effective dose, since
966   increasing the dose from C to D did not further increase survival. Ideally, the
967   exposures in animals resulting from the administration of Dose C should serve as
968   the reference point for comparison with human exposures, but there is uncertainty
969   as to whether the E/R relationship in humans is similar to the E/R relationship in
970   animals. The dose and regimen for humans should be selected to provide
971   exposures that exceed those associated with the fully effective dose in animals,
972   ideally by several-fold, based on knowledge of this reference point, the drug's
973   safety profile, and human PK data at the agreed upon upper limit for human
974   dosing. To minimize the possibility of sub-therapeutic exposures, human dose
975   selection should also take into account the variability of exposure parameters in
976   humans and healthy and affected animals so that any low outlying values of
977   exposure in humans will be greater than those associated with efficacy in animals.
978

979
980   **Figure 2   Comparisons of Animal and Human PK Data to Support the**
981   **Selection of an Effective Dose in Humans**
982

983
984
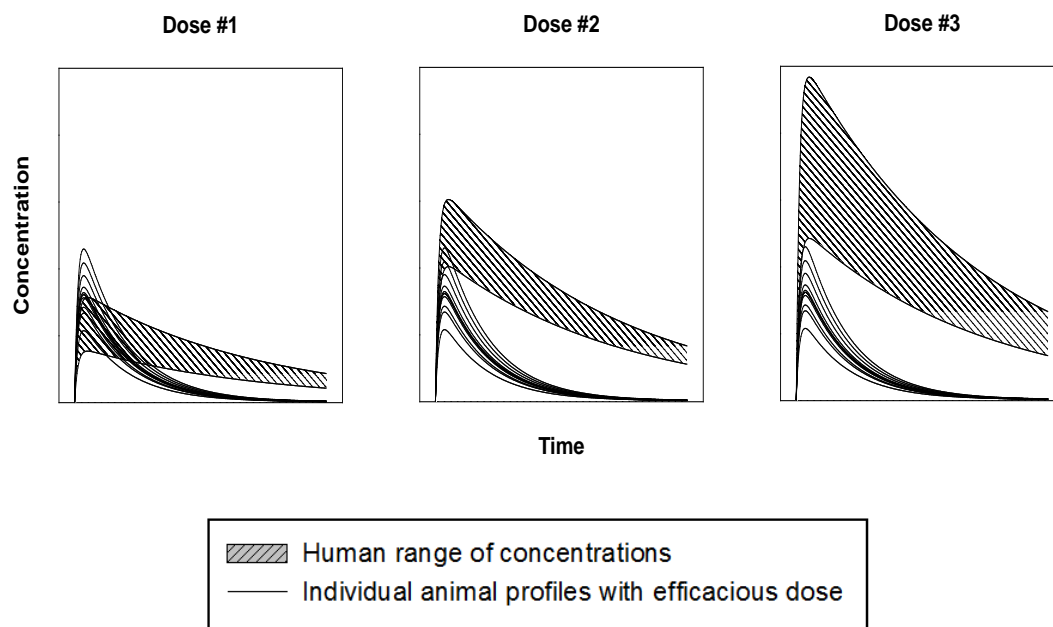
987   In Figure 2, ranges of systemic drug concentration-versus-time profiles from
988   human subjects following administration of three well-tolerated doses of an
989   investigational drug are superimposed on the systemic concentration profiles from
990   individual animals administered a fully effective dose. Based on a comparison of
991   the animal and human PK data, Dose 3 represents an ideal situation with the full
992   range of human exposures exceeding the exposures for each animal administered
993   the fully effective dose, both for Cmax and overall exposure. If efficacy is not
994   associated with the drug's Cmax, Dose 2 also represents an ideal situation. In the
995   absence of scientific justification, Dose 1 is not acceptable because the full range
996   of human exposures is not greater than the exposures associated with efficacy in
997   animals.
998
999   Interspecies differences in ADME should be considered when determining the
1000   human dose. Differences in ADME between animals and humans may result in
1001   different systemic concentration-versus-time profiles among species,[67,68] that may
1002   necessitate adjustments in the dose or regimen tested in the adequate and well-
1003   controlled animal efficacy studies to achieve concentration-versus-time profiles
1004   that are similar to the profile observed in humans. Failure to account for

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[67] Deziel, MR, et al., 2005, Effective Antimicrobial Regimens for Use in Humans for Therapy of *Bacillus anthracis* Infections and Postexposure Prophylaxis, *Antimicrob Agents Chemother*, 49(12):5099-5106.

[68] Kao, LM, et al., 2006, Pharmacokinetic Considerations and Efficacy of Levofloxacin in an Inhalational Anthrax (Postexposure) Rhesus Monkey Model, *Antimicrob Agents Chemother*, 50(11):3535-3542.

1005   interspecies differences in PK may result in exposures in animals that are not
1006   achievable in humans and the inability to select an effective dose in humans (see
1007   section VI.B for additional discussion). Differences in protein binding
1008   characteristics between animals and humans also should be considered, because
1009   only free drug, or the unbound fraction, is pharmacologically active. If the
1010   protein binding characteristics in the selected species differ from those of humans,
1011   comparison of free drug exposures will be relevant for dose selection.

1012
1013   Although not discussed further in this document, quantitative methods, such as
1014   conventional PK modeling or physiologically-based pharmacokinetic (PBPK)
1015   modeling, can be used to support the extrapolation of exposures in animals to
1016   doses in humans. The use of such methods should be discussed with the review
1017   division.

1018
1019   Sponsors should consider PK interactions in humans of the investigational drug
1020   with medical products likely to be used concomitantly in the clinical scenario.
1021   The sponsor, with knowledge of the ADME of the investigational drug, should
1022   discuss with FDA other medical products that are likely to be co-administered
1023   based on the clinical scenario and develop a plan to address the potential for
1024   human PK interactions using in vitro and in vivo assessments, if warranted.
1025   Potential combinations that may affect the PK of either drug should be considered
1026   for interaction studies. For example, if the investigational drug is metabolized via
1027   the cytochrome P450 system (CYP450), the safety or efficacy of the
1028   investigational drug can be compromised by the concomitant use of CYP450
1029   inhibitors or inducers, and such drug-drug interactions should be evaluated. In the
1030   case of therapeutic biological products, the design and conduct of relevant drug-
1031   biologic interaction studies should be discussed with FDA with the overall goal of
1032   determining interactions with clinical impact.

1033
1034   When PD-based interactions (i.e., non-ADME based synergy or antagonism) with
1035   other drugs likely to be used in the anticipated clinical scenario have been
1036   identified, the sponsor should discuss with FDA the potential impact of these
1037   findings on the final human dose selection. For further discussion, see section
1038   VI.A, below.

1039
1040
1041 **VI.   DESIGN CONSIDERATIONS FOR THE ADEQUATE AND WELL-**
1042   **CONTROLLED EFFICACY STUDIES IN ANIMALS**
1043
1044 The assessment of efficacy in animals should follow best practices for adequate and well-
1045 controlled human efficacy studies, with endpoints that demonstrate an important clinical benefit,
1046 generally the enhancement of survival or prevention of major morbidity. If a well-characterized
1047 animal model in a single species is used, FDA may require a confirmatory animal efficacy study

1048   in that animal model.[69]   Conduct of the confirmatory study at a different laboratory will support
1049   the robustness of the findings. Supportive human efficacy data in a related indication may negate
1050   the need for a confirmatory study. Early discussions between the sponsor and FDA about study
1051   design (including protocol, endpoints, proposed statistical analysis plan, and data quality and
1052   integrity plan if specific aspects of the study are anticipated to be challenging with regard to
1053   GLP) and study conduct are highly recommended. Agreement on these issues should be reached
1054   before the initiation of studies.
1055
1056   **A.   General Principles**
1057
1058   Studies should be designed to mimic the ultimate clinical use of the investigational drug and to
1059   achieve meaningful outcomes similar to the benefits desired in humans. The animal studies
1060   should not use surrogate endpoints[70] as the sole evidence of efficacy. It is unlikely that surrogate
1061   endpoints will be persuasive to FDA because the Animal Rule requires that the animal study
1062   endpoint (generally, decrease in mortality or reduction in significant morbidity) be clearly related
1063   to the clinical benefit.[71]   Analyses of secondary endpoints may contribute to an understanding of
1064   the disease or condition and a characterization of the treatment effect.
1065
1066   With rare exceptions, the adequate and well-controlled animal efficacy studies should evaluate
1067   the E/R relationship of the investigational drug, unless earlier studies have established the
1068   effective dose. For further discussion of dose selection in the animals, see section VI.B. The
1069   study duration is determined by the endpoint selected for the proposed indication. The study
1070   duration should incorporate adequate follow-up time to observe for recurrence of disease or
1071   condition after stopping drug administration. The route of administration of the investigational
1072   drug in animals should be the same as the route in humans, unless adequate justification is
1073   provided. Different dosing regimens in animals and humans may be needed to provide
1074   comparable exposure to the drug.
1075
1076   Animals of both sexes should be included. FDA recognizes that there are significant supply
1077   constraints on the use of adult animals of certain species. The sponsor should discuss the age
1078   and the immune status of the animals used in efficacy studies, as compared to the intended
1079   human population. Inclusion and exclusion criteria for the acceptance of the animals into the
1080   study should be appropriate and pre-specified before initiating the studies.
1081
1082   The time course of observation should be optimized to assess the true treatment effect and to
1083   detect possible adverse effects. Animals should be monitored frequently; the frequency of

---

[69] As stated in the preamble to the final rule, "…the animal studies should be replicated or substantiated in each species as needed to ensure credible results…" (67 *Federal Register* 37988 at 37991, May 31, 2002).

[70] In this context, the term, *surrogate endpoint*, refers to a surrogate endpoint for efficacy (i.e., a drug-induced change in a biomarker that is considered reasonably likely to predict the clinical benefit of the drug; for example, decreased viral load or increased neutrophil count) (see 21 CFR 314.510, subpart H for drugs and 21 CFR 601.41, subpart E for biological products). Surrogate endpoints for efficacy are conceptually distinct from *humane endpoints.* Prospectively defined, objective euthanasia criteria that are necessary to address animal welfare are based on the selected humane endpoints (see Appendix A).

[71] See 21 CFR 314.610(a)(3) for drugs; 21 CFR 601.91(a)(3) for biological products.

1084 observation may vary over the course of the study depending on the actual mechanism of disease
1085 or injury. In these studies that use mortality or major morbidity as an endpoint, observation
1086 frequency should be sufficient to ensure animal welfare and to minimize the potential loss or
1087 compromise of data.
1088
1089 Prospectively designed statistical analysis plans should be developed, incorporating the
1090 appropriate levels of statistical significance, including descriptions of the randomization
1091 procedures and methods to address missing data and, if applicable, outlying data. Protection
1092 against bias is critical in animal studies, just as it is in human trials. Studies should be
1093 randomized, and given that these animal studies are frequently small in size, variable block
1094 randomization is preferable to minimize bias. Euthanasia criteria should be prospectively
1095 specified and sponsors should provide a discussion of the potential effects of the criteria on the
1096 interpretation of results. Studies should be blinded, including blinded reading of histopathology
1097 slides, and study procedures should be applied uniformly to all study groups. Any situation in
1098 which study personnel may become aware of treatment assignments should be discussed with
1099 FDA in advance because of the potential for major effects on study interpretability.
1100
1101 For almost any situation in which the Animal Rule might be used, there will be no basis for
1102 relying on a non-inferiority study to support effectiveness, and placebo-controlled animal studies
1103 should be used to demonstrate effectiveness. Data obtained in the placebo-control group of the
1104 efficacy study should be compared with the data obtained in the natural history or model
1105 characterization studies to substantiate the animal model. For example, if animals in the
1106 placebo-control group do not exhibit morbidity or mortality similar to that seen in the natural
1107 history studies, this may reflect a problem with preparation of the challenge substance that limits
1108 the ability to interpret outcomes in the active treatment arm(s) of the study.
1109
1110 If a drug has already been approved for the same indication and approval was based on the same
1111 animal species in which the investigational drug is being evaluated, the use of the approved drug
1112 in an active comparator arm, in addition to the investigational drug and placebo arms, is
1113 encouraged and should be discussed with the review division. The inclusion of the active
1114 comparator can test for assay sensitivity (i.e., the ability of the study to differentiate an effective
1115 drug from an ineffective drug).
1116
1117 Investigational drugs should be evaluated within the context that reflects anticipated clinical
1118 use.[72] When appropriate, supportive care similar to what would be expected to be provided in
1119 humans should be used for the animals[73] (see Appendix B for further discussion). When
1120 supportive care is used, the study should show that the investigational drug with supportive care
1121 is superior to placebo with supportive care. When incorporated into a study, supportive care

---

[72] The need for supportive care should be directed by the concept of operations (i.e., how the product will be used during an incident).

[73] When it is anticipated that supportive care will be used in the adequate and well-controlled animal efficacy studies, the assessment of similar supportive care in model development, including the natural history studies used to define the model, should be discussed with the review division.

1122 should be administered either to all animals on a set schedule or to individual animals according
1123 to prospectively defined triggers, based on preliminary studies or available literature. When
1124 supportive care will be administered to individual animals based on prospectively designed
1125 treatment triggers, the statistical plan should take into account the potential impact on the
1126 efficacy endpoint of differing supportive care among animals. The potential effects of the
1127 supportive care on the animal and on the PK and/or PD of the investigational drug should be
1128 considered in the design and interpretation of the study.
1129
1130 In addition, the sponsor, in consultation with FDA, should consider other drugs that are likely to
1131 be used and evaluate whether the activity of either drug, when used in combination, is affected
1132 by PD-based interactions (i.e., non-ADME based synergy or antagonism) and develop a plan to
1133 address the potential for such interactions. For example, it should be known whether the use of
1134 an anthrax antitoxin monoclonal antibody will have an effect on the activity of the antimicrobial
1135 drugs used for the treatment of disseminated anthrax disease, or whether the use of a drug that
1136 prevents replication of the target organism, resulting in a diminished immune response, may
1137 decrease the efficacy of a vaccine against that organism.
1138
1139 A checklist of elements of an adequate and well-controlled animal efficacy study protocol is
1140 provided in section X. In general, FDA should have the opportunity to review information on
1141 the proposed clinical indication, animal model, and method to be used to translate the effective
1142 exposures in animals to a dose and regimen in humans prior to detailed discussions regarding the
1143 design of a specific adequate and well-controlled efficacy study in animals. The design of an
1144 animal efficacy study should incorporate the principles discussed in sections IV and V.
1145 Protocols for these studies can be submitted with a request for review under the SPA provisions
1146 (see section III.A).
1147
1148   **B.**   **Dose Selection in Animals**
1149
1150 The selection of the doses of the investigational drug[74,75] to be studied in the adequate and well-
1151 controlled animal efficacy studies should be based on an understanding of the E/R relationship in
1152 the proposed animal model. Dose range-finding studies should include at least three adequately
1153 spaced doses to help define the shape of the E/R curve, including establishing a fully effective
1154 dose (see Figure 1 in section V.B.2.b). To identify a fully effective dose, it is generally useful to
1155 have studied a higher dose and shown no added benefit. For example, in Figure 1, the survival
1156 demonstrated with Dose D confirms that Dose C is a fully effective dose. At least one of the
1157 doses evaluated in the adequate and well-controlled efficacy studies should be a fully effective
1158 dose.
1159
1160

---

[74] This discussion assumes that the investigational drug is the active moiety. Issues related to active metabolites are handled on a case-by-case basis and should be discussed with the review division.

[75] For information on preventive vaccine dose selection see section VII.A.

1161 Prior to selecting doses for the efficacy studies, sponsors should understand the differences in
1162 ADME between humans and the selected animal species. Differences in ADME between
1163 animals and humans may result in different systemic concentration-versus-time profiles between
1164 species.[76,77] Failure to account for PK differences among species may result in exposures in
1165 animals that are not achievable in humans and, thus, the inability to select an effective dose in
1166 humans. Some differences in systemic concentration-versus-time profiles between animals and
1167 humans may necessitate adjustments of dose regimens studied in animal efficacy studies to
1168 achieve concentration-versus-time profiles that are similar to the profile observed in humans.
1169 This is known as "humanization" of dose regimens and it is illustrated in Figure 3.
1170
1171 **Figure 3   An Example of a "Humanized" Dose and Regimen for Evaluation in**
1172 **an Animal Model of Disease[78]**
1173
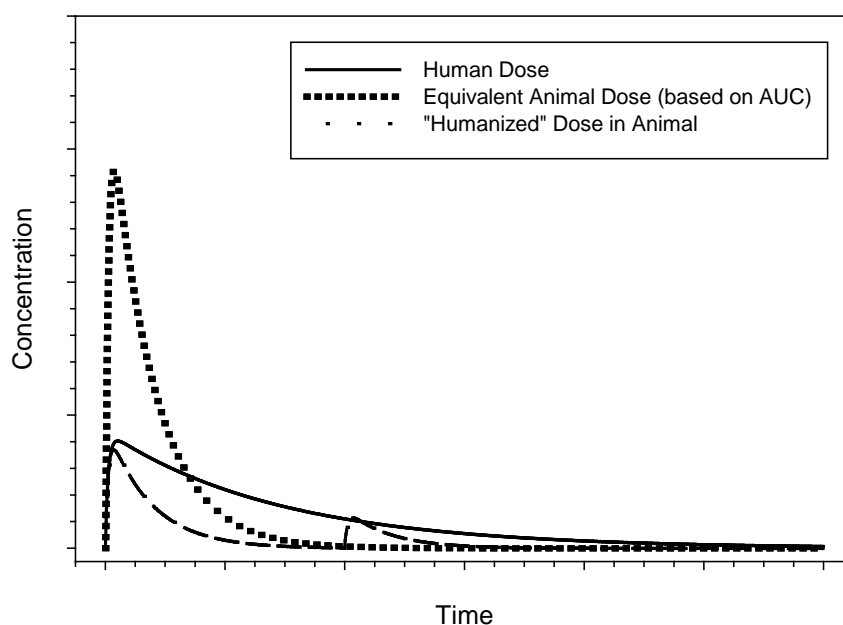
1174
1175 In this example, the shapes of the animal and human exposure profiles following once daily
1176 dosing are not comparable because the half-life of the drug in animals is much shorter than in
1177 humans. The dose regimen in animals is manipulated to achieve an exposure profile that is more
1178 similar in shape to that of humans. Adjusting the dose regimen used in animal studies based on
1179 differences in pharmacokinetics enables an improved comparison of exposures between animals
1180 and humans and, thus, greater confidence in selecting an effective dose in humans.
1181

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[76] Deziel, MR, et al., 2005, Effective Antimicrobial Regimens for Use in Humans for Therapy of *Bacillus anthracis* Infections and Postexposure Prophylaxis, *Antimicrob Agents Chemother*, 49(12):5099-5106.

[77] Kao, LM, et al., 2006, Pharmacokinetic Considerations and Efficacy of Levofloxacin in an Inhalational Anthrax (Postexposure) Rhesus Monkey Model, *Antimicrob Agents Chemother*, 50(11):3535-3542.

[78] Adapted from Bergman, KL, 2009, The Animal Rule and Emerging Infections: The Role of Clinical Pharmacology in Determining an Effective Dose, *Clin Pharmacol Ther*, 86 (3):328-331.

1182
1183 **VII. CONSIDERATIONS FOR PREVENTIVE VACCINES AND FOR CELLULAR**
1184 **AND GENE THERAPIES**
1185
1186 Although the overall principles of this guidance are applicable to vaccines[79] and to cellular and
1187 gene therapy products, additional considerations in the design of the animal efficacy studies exist
1188 because of the biological nature of these products. This section describes general considerations
1189 for study design and selection of relevant animal species for the adequate and well-controlled
1190 animal efficacy studies specific to vaccines and to cellular and gene therapy products. Before
1191 conducting an adequate and well-controlled animal efficacy study, FDA recommends that a
1192 sponsor request a meeting to discuss the details of the animal model(s) and study design,
1193 including the rationale and methods that will be used to extrapolate from a dose level(s) that
1194 shows substantial benefit in the animal studies to the final human dose and regimen.
1195
1196   **A.   Vaccines**
1197
1198 FDA will rely on animal efficacy data for approval of vaccines using the Animal Rule only when
1199 the animal study endpoint is clearly related to the desired benefit in humans, generally the
1200 enhancement of survival or the prevention of major morbidity. To estimate efficacy of vaccines
1201 in humans using the Animal Rule, the vaccine dose chosen for adequate and well-controlled
1202 animal efficacy studies should elicit an immune response in animals reflective of that in humans.
1203 Using pilot and proof-of-concept studies, a relationship should be established between the
1204 vaccine dose and the desired immune response, depending upon the study endpoint. The dose,
1205 route of immunization, and schedule may be different in the animal and human studies if the
1206 relevant immune response is similar, and adequate justification is made.
1207
1208 Sponsors should develop an approach for bridging animal responses to humans by careful
1209 selection of appropriate immune markers. Sponsors should accumulate as much immune
1210 response data as possible in their animal model(s), sufficient to characterize the immune
1211 response that is associated with the desired outcome of disease prevention. Such data may be
1212 used to establish the vaccine dose in humans necessary to induce analogous immune responses.
1213 The animal immune response should reflect the response achieved by humans and support the
1214 selection of an effective human dose and immunization schedule. Sponsors should discuss with
1215 FDA their choice of an immune marker, which will depend upon the product and the animal
1216 model selected for these studies.
1217
1218 A single immune marker in an animal model may not reflect the spectrum of protective immune
1219 responses generated by humans. For example, for certain intracellular pathogens, animal models
1220 should be selected that demonstrate the induction of a protective antibody response as well as
1221 novel cellular immune response markers similar to humans. The choice of animal species should
1222 be made based on consultation with experts, review of the literature, discussions at scientific
1223 workshops and meetings, and discussions with FDA.
1224

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[79] Cancer vaccines and therapeutic vaccines for non-infectious diseases are outside the scope of this guidance.

1225 The challenge agent used in animal studies with vaccine products should be relevant to the
1226 human disease. When the etiologic agent's host range prevents the development of an
1227 acceptable animal model, studies may be conducted in animal models with closely related
1228 challenge strains, assuming that cross strain immune markers, such as cross reacting neutralizing
1229 antibodies, allow bridging to the human immune response. Ideally, the animal model(s) should
1230 show similar pathophysiology, progression of disease, symptoms, and host immune response to
1231 that observed in humans. Achieving this may call for optimization of the animal models in pilot
1232 and proof-of-concept studies using variable doses of the challenge agent to allow evaluation of
1233 the product's effectiveness and interpretation of the study endpoints in the adequate and well-
1234 controlled animal efficacy study(ies). Ideally, the route of exposure should reflect the
1235 anticipated route of human exposure (especially if the route of exposure significantly affects the
1236 pathophysiology, onset, and progression of disease). However, when the natural route of
1237 exposure is not known or cannot be replicated in a model, animal studies to demonstrate
1238 protective immune responses using other routes of exposure may be considered and should be
1239 discussed with FDA. Appropriate animal efficacy studies should be designed to provide
1240 information about the duration of protection afforded by the vaccine.
1241
1242 Sponsors should seek and carefully consider guidance from public health officials and experts
1243 concerning the intended use of the vaccine product. Either or both pre- and post-exposure
1244 prophylaxis clinical indications may be desired depending upon public health needs. Important
1245 immunization parameters, including the optimal dose, schedule, and the desired time and
1246 duration of protection, may differ depending upon the indication. Studies supporting post-
1247 exposure use may be more technically challenging to design depending upon the animal model.
1248 Vaccines used in post-exposure scenarios would be expected to be given as soon as an exposure
1249 is recognized and should induce an immune response in animal models that can be extrapolated
1250 to humans and suggest clinical benefit. Data derived from pre-exposure prophylaxis studies may
1251 support the design of post-exposure animal studies, especially with regard to the kinetics and
1252 peak of the immune response. Sponsors should evaluate the possible concomitant use and
1253 resulting influence of therapeutic drugs and antibiotics on effectiveness of the product when
1254 designing studies of vaccines intended for use in post-exposure scenarios.
1255
1256 **B.   Cellular and Gene Therapies**
1257
1258 *1.   Cellular Therapy Products*
1259
1260 The selection of relevant animal species for evaluation of a cellular therapy product
1261 should include consideration of the host animal's response to the product, including
1262 inflammatory responses, innate and acquired immune responses, and interactions of the
1263 cells with the host (direct and indirect biological responsiveness).[80] In addition, in vivo
1264 cell fate following delivery using the clinical route of administration should be
1265 characterized in each species. Cell fate includes cell distribution to target and non-target
1266 sites, survival/engraftment, differentiation and integration, phenotype, and proliferation.

---

[80] For a more comprehensive discussion of the overall principles for the cellular and gene therapy products, refer to FDA's guidance for industry *Preclinical Assessment of Investigational Cellular and Gene Therapy Products.*

1267   Administration of the cellular therapy product to healthy animals will not likely result in
1268   data representative of cell fate in humans. For example, in GI-ARS, cell turnover and
1269   mitotic rate will affect cell fate; thus, the response of the crypt cells to the cellular therapy
1270   pre- and post-radiation exposure will not be the same. In addition, if the cellular therapy
1271   product is delivered in combination with a matrix and/or scaffold or in an
1272   immunoisolation device, the biodegradation profile of these constructs should also be
1273   characterized.
1274
1275   If the cell fate, cell function, and/or host response to the cells in the animal species differs
1276   greatly from what is known or predicted in humans, administration of a well-
1277   characterized analogous cellular product[81] in the animal studies may be considered. The
1278   use of an analogous cellular product in an animal efficacy study is predicated on the
1279   ability to identify, harvest, and characterize (e.g., phenotyping and potency) a similar cell
1280   population in the animal species used for testing. Production of the analogous cellular
1281   product should meet the same standards as those applied to production of the final human
1282   cellular therapy product. Sponsors are encouraged to initiate discussions with FDA early
1283   in product development for guidance on the animal models and the potential use of an
1284   analogous cellular product prior to initiating the adequate and well-controlled efficacy
1285   studies.
1286
1287   *2.*   *Gene Therapy Products*
1288
1289   The selection of relevant animal species for evaluation of a gene therapy product should
1290   include consideration of the host animal's response to the clinical vector, the expressed
1291   transgene, and/or the genetically modified cells.[82] Vector-specific issues include
1292   determining (1) the permissiveness and/or susceptibility of various animal species to
1293   infection and replication by the viral vector, (2) if an immune or inflammatory response
1294   develops against the vector and the effect of the response on the in vivo expression and
1295   persistence of the vector, (3) if an immune response develops against vector positive
1296   cells, and (4) if pre-existing immunity to the vector exists in the animals.
1297
1298   Transgene-specific issues include determining (1) the pharmacological response of the
1299   species to the expressed transgene, (2) whether an immune or inflammatory response to
1300   the expressed transgene and/or protein develops, and (3) if an immune or inflammatory
1301   response does develop, the effect of the response on the in vivo expression levels,
1302   persistence, and functionality of the expressed transgene and/or protein in the animal
1303   species. If these transgene-specific factors significantly differ in the animal species from
1304   what is known or predicted in human cells and tissues, administration of the clinical
1305   vector modified to express an analogous transgene[83] may be considered. In such

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[81] As used in this guidance, *analogous cellular products* are defined as cellular products derived from the animal species used for testing that are analogs of the ultimate clinical product in phenotype and biologic activity.

[82] For a more comprehensive discussion of the overall principles for the cellular and gene therapy products, refer to FDA's guidance for industry *Preclinical Assessment of Investigational Cellular and Gene Therapy Products.*

[83] As used in this guidance, an *analogous transgene* is defined as a transgene derived from the animal species used for testing that is an analog of the human derived transgene in the clinical vector.

1306   instances, product characterization comparison between the intended clinical construct
1307   and the animal homolog should be provided.

1308

1309   Issues related to genetically modified cells include (1) the sensitivity of the species to the
1310   biological actions of the modified cells and (2) the considerations conveyed in section
1311   VII.B.1.

1312

1313

1314 **VIII.   HUMAN SAFETY INFORMATION**

1315

1316 The Animal Rule neither replaces the need, nor establishes special requirements, for an adequate
1317 human safety database for drug development. The expectation is that drugs "will be evaluated
1318 for safety under preexisting requirements for establishing the safety of new drug and biological
1319 products."[84] FDA anticipates that the nonclinical and clinical safety development programs will
1320 proceed in a manner similar to that of drugs developed under traditional regulatory pathways.
1321 Some of the general principles include the following:

1322

1323   •   Nonclinical toxicology, safety pharmacology, and PK studies should provide adequate
1324   safety data to support the initiation of human trials.

1325   •   Risk-benefit assessment and ethical considerations must guide the design of human trials
1326   at each phase of development.[85] The regulatory and ethical complexities of establishing
1327   the necessary safety database should be discussed with the review division, preferably
1328   early in the drug development program.

1329   •   The size and composition of the human safety database should be consistent with the
1330   proposed use of the drug.

1331   •   The adverse event grading scale should be appropriate for the population to be studied
1332   (e.g., healthy adult human volunteers[86]).

1333   •   Safety signals identified from animal studies or human trials should be characterized and,
1334   if necessary, specific study design elements should be incorporated into the proposed
1335   nonclinical and/or clinical protocols to prevent or mitigate toxicity in future studies.

1336

1337 The evolving safety profile of the drug may necessitate changes in the clinical development
1338 program. When evaluating the available human and animal data at key steps during drug
1339 development, sponsors should determine whether the program remains on a suitable path to
1340 achieve an adequate human safety database and consult with FDA if necessary.

1341

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[84] See 67 *Federal Register* 37988 at 37989, May 31, 2002.

[85] See protection of human subjects regulations at 21 CFR 50 and institutional review boards regulations at 21 CFR 56.

[86] The principles expressed in the following FDA guidance for industry may be useful: *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.*

1342   When the potential for benefit to individual human subjects in studies of drugs being developed
1343   under the Animal Rule is remote, the risks must be carefully considered. Even a compelling
1344   need for a drug (e.g., natural disaster, national threat) does not in itself justify exposing study
1345   subjects to risks greater than those acceptable for other drug development programs. For drugs
1346   with only minor anticipated risks, studies in competent, appropriately consented adults are
1347   considered reasonable.[87] If concerns about safety and/or relevance limit the extent or usefulness
1348   of studies in healthy adult volunteers, sponsors should explore alternative approaches to
1349   contribute to the aggregate safety database. In some cases, studies can be conducted in existing
1350   patient populations for whom development of the drug might fill a need (even if the population is
1351   different from the intended target population) or existing safety data already may be available.
1352   For example, the safety information used to support levofloxacin's pneumonic and septicemic
1353   plague indications was obtained from the large safety database from its other approved
1354   indications.
1355
1356   The necessary overall size and composition of the human safety database depend on issues such
1357   as the proposed indication, the drug's toxicity, and/or the extent of FDA's experience with a
1358   particular drug class. If the drug of interest is already approved, some of the existing safety data
1359   may be relevant to the proposed Animal Rule indication. Similarly, if the drug of interest is in
1360   development for another indication, accrued safety data may be relevant for the proposed Animal
1361   Rule indication.
1362
1363   The numbers suggested below refer to individuals exposed to the proposed route of
1364   administration, dosage form, formulation, and, at a minimum, the proposed dose, regimen, and
1365   duration. For a drug intended for the treatment of a specified life-threatening disease or
1366   condition, greater known risks or greater uncertainty about undefined risks may be acceptable
1367   when the drug offers a clear benefit for those patients. In most cases, a database of at least 300
1368   individuals would be needed for a 95% confidence interval to rule out a 1% rate of a specific
1369   adverse reaction (e.g., liver failure) if that specific adverse reaction did not occur in the
1370   population studied. In contrast, drugs intended for prophylaxis in large numbers of healthy
1371   persons with variable or unclear risk of disease or injury may require a safety database in the
1372   thousands to facilitate an adequate risk-benefit assessment because little if any toxicity risk or
1373   undefined risks will be acceptable in this population. If a drug has a known high risk of serious
1374   or life-threatening adverse reactions, the risk-benefit analysis may be deemed unacceptable for
1375   proceeding with healthy volunteer studies. In this case, if the sponsor believes a drug might still
1376   offer an acceptable risk-benefit in a specified emergency situation, discussion with FDA should
1377   be initiated to determine whether a path forward is identifiable.
1378
1379   Other safety considerations include the potential for interactions, such as between drugs (e.g., a
1380   colony-stimulating factor and another investigational drug that modifies the host immune
1381   system) or between the drug and a disease (pre-existing or agent-induced). Animal models used
1382   to demonstrate efficacy may not predict specific interactions of the agent-induced disease or
1383   condition and the investigational drug in humans. Adverse interactions in humans may not be

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[87] As stated in 21 CFR 56.111(a)(2), "Risks to subjects are reasonable in relation to anticipated benefits, if any, to
subjects, and the importance of the knowledge that may be expected to result."

1384 observed until the drug is used for the disease or condition, reinforcing the critical need for
1385 postmarketing studies.[88] If adverse findings occur only when the investigational drug is tested
1386 in challenge agent-affected animals, further investigation may be warranted to determine the
1387 pathophysiological mechanism for the unexpected toxicity and its relevance to the risk
1388 assessment for the intended human population.
1389

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[88] Postmarketing studies to provide evaluation of safety and efficacy in the event an emergency arises and the product is used are required under the Animal Rule when such studies are feasible and ethical. A plan or approach for conducting such trials must be included with the NDA or BLA (for greater detail, see 21 CFR 314.610(b)(1) for drugs and 601.91(b)(1) for biological products).

1390
1391 **IX.   CHECKLIST OF ESSENTIAL ELEMENTS OF AN ANIMAL MODEL**
1392
1393   The following checklist provides a list of data elements (and the corresponding sections within
1394   this guidance) for consideration when developing an animal model. The purpose of this
1395   checklist is to remind sponsors of the need to compare the data elements for the selected animal
1396   species to what is known about the human disease or condition in their submissions to FDA.
1397   Sponsors should note and explain any differences and indicate if they expect these differences to
1398   have an impact on the interpretability of the data.
1399

DATA ELEMENTS (Corresponding Sections Within the Guidance)	Animal(s)	Human
ELEMENTS RELATED TO THE ETIOLOGIC OR CHALLENGE AGENT-INDUCED DISEASE OR CONDITION		
CHARACTERISTICS OF THE ETIOLOGIC OR CHALLENGE AGENT		
• The Challenge Agent (V.A.1.a)		
• Pathophysiological Mechanisms of Toxicity or Virulence (V.A.1.b)		
• Route of Exposure (V.A.1.c)		
• Dose and Quantification of Exposure (V.A.1.d)		
HOST SUSCEPTIBILITY AND RESPONSE (V.A.2)		
NATURAL HISTORY OF THE DISEASE OR CONDITION - PATHOPHYSIOLOGICAL COMPARABILITY		
• Time to Onset (V.A.3.a)		
• Time Course of Progression (V.A.3.b)		
• Manifestations (V.A.3.c)		
TRIGGER FOR INTERVENTION (V.A.4)		
ELEMENTS RELATED TO THE INVESTIGATIONAL DRUG AND THE SELECTION OF AN EFFECTIVE DOSE IN HUMANS		
THE INVESTIGATIONAL DRUG		
• Mechanism of Action (V.B.1.a)		
• Drug Class (V.B.1.b)		
• Dosage Form and Route of Administration (V.B.1.c)		
SELECTION OF AN EFFECTIVE DOSE IN HUMANS (‡)		
• PK and PD Information to Be Obtained in Animals and Humans (V.B.2.a)		
• PK/PD Considerations for Human Dose Selection (V.B.2.b)		
(‡) For information on vaccine dose selection see section VII.A.		

1400

1401
1402 **X. CHECKLIST OF ELEMENTS OF AN ADEQUATE AND WELL-CONTROLLED**
1403 **ANIMAL EFFICACY STUDY PROTOCOL**
1404
1405 This checklist is included to remind sponsors of the information that should be included in their
1406 adequate and well-controlled animal efficacy study protocols. For further information, refer to
1407 section VI.
1408

PROTOCOL CONSIDERATIONS		
• Indication to Be Studied		
• Agency Concurrence on the Details of the Animal Model		
• Comparability of the Study Design to the Clinical Scenario		
STUDY DESIGN ELEMENTS	**Described**	**Justified**
• Controls		
• Size of Study Groups and Male/Female Composition of Groups		
• Animal Characteristics (†) (e.g., species, age, weight, source of animals)		
• Inclusion and Exclusion Criteria for Acceptance Into Study		
• Dose, Route of Exposure, and Preparation of the Challenge Agent		
• Trigger for Intervention		
• Dose, Regimen, and Route of Administration of the Investigational Drug		
• Randomization		
• Blinding		
• Statistical Plan		
• Endpoints		
• Euthanasia Criteria		
• Observation Frequency and Schedule		
• Animal Care Interventions		
• Plan for Ensuring the Quality and Integrity of the Data		
(†) See section IV.D for further description		

1409
1410 **APPENDIX A: GENERAL PRINCIPLES FOR THE CARE AND USE OF ANIMALS IN**
1411 **BIOMEDICAL RESEARCH**
1412
1413 Animal studies conducted in the United States and its territories must comply with applicable
1414 laws and regulations as prescribed by the Animal Welfare Act[89] and the Public Health Service
1415 Policy on Humane Care and Use of Laboratory Animals.[90]
1416
1417 The following statements summarize general principles for the care and use of animals in
1418 biomedical research based on the animal welfare references listed at the end of this Appendix:
1419
1420   1. All persons involved in the use of animals in biomedical research should be appropriately
1421   qualified for and experienced in conducting procedures on living animals.
1422
1423   2. The living conditions of animals should be appropriate for the species and contribute to
1424   their health and comfort.
1425
1426   3. Unless otherwise established, procedures that cause pain or distress in human beings
1427   should be considered to cause pain or distress in animals. For such procedures, the
1428   following practices should be observed, unless there is compelling scientific reason
1429   precluding such practices:
1430   a. Appropriate sedation, analgesia, or anesthesia should be used during and/or
1431   following procedures that may cause more than momentary or slight pain or
1432   distress.
1433   b. Humane endpoints that do not jeopardize the scientific objectives of the study
1434   should be established to prevent animals from suffering unrelieved pain or
1435   distress.[91] Humane endpoints are the earliest indicators of severe distress, severe
1436   pain, suffering or impending death observed in an experimental animal.[92]
1437   Predetermined humane endpoints are used to develop objective euthanasia
1438   criteria. Research necessitating endpoints for which pain and distress are not
1439   alleviated needs to be justified to, and approved by, the Institutional Animal Care
1440   and Use Committee (IACUC).[93]

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[89] See 7 U.S.C. 2131 et seq.

[90] National Institutes of Health, Office of Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," 2002, http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on November 21, 2013.

[91] Humane endpoints are conceptually distinct from surrogate endpoints for efficacy. Surrogate endpoints for efficacy are discussed in section VI.A.

[92] Organisation for Economic Co-operation and Development, 2000, Guidance Document on the Recognition, Assessment, and Use of Clinical Signs as Humane Endpoints for Experimental Animals Used in Safety Evaluation, ENV/JM/MONO(2000)7, OECD, Paris, France.

[93] See 9 CFR 2.31(d)(iv)(A).

1441   c.   Animals experiencing severe or chronic pain or distress that cannot be relieved
1442   should be euthanized painlessly. The appropriate use of euthanasia criteria is
1443   beneficial to the animal because unnecessary terminal distress is eliminated or
1444   significantly reduced. Also, it benefits the research effort because experimental
1445   goals can be met more consistently. Data collected after the development of
1446   severe physiologic derangements may not be useful or may be misleading for
1447   some purposes. Also, tissues that might otherwise be lost can be collected for
1448   postmortem analysis. Prospectively defined criteria for euthanasia should be
1449   included in protocol development. The criteria should be predictive of imminent
1450   death or specific moribund conditions and should be defined in objective terms
1451   that are relevant to the specific experiment.

1452   d.   For studies in which major morbidity or mortality are expected, observation
1453   frequency should be increased around the expected time of major morbidity or
1454   death to prevent animals from experiencing unrelieved pain or distress and also to
1455   minimize the potential compromise or loss of data.

1456

1457   4.   Adequate veterinary oversight and care provided by a qualified veterinarian, as defined
1458   by the Animal Welfare Act, and involvement of the IACUC must be in place to ensure
1459   humane care and use of animals. [94,95,96] The attending veterinarian and IACUC should
1460   play an active role in providing advice on humane endpoints and adequate veterinary care
1461   necessary to ensure the humane needs of animals are met and are compatible with the
1462   scientific requirements of the study.

1463

1464 Animal welfare references include:

1465   •   The Animal Welfare Act[97]

1466   •   Guide for the Care and Use of Laboratory Animals, 8th edition[98]

1467   •   Public Health Service Policy on Humane Care and Use of Laboratory Animals[99]

1468

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[94] See 7 U.S.C. 2131 et seq.

[95] See Health Research Extension Act of 1985, Public Law 99-158.

[96] See 9 CFR 2.31 and 9 CFR 2.33.

[97] See 7 U.S.C. 2131 et seq.

[98] National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011, *Guide for the Care and Use of Laboratory Animals*, 8th edition, Washington, DC: National Academies Press (US).

[99] National Institutes of Health, Office of Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," 2002, http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on November 21, 2013.

1469

1470   • U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used
1471   in Testing, Research and Training[100]

1472   • AVMA Guidelines for the Euthanasia of Animals, 2013 edition[101]

1473   • Recognition and Alleviation of Pain in Laboratory Animals[102]

1474

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[100] See 50 *Federal Register* 20864, May 20, 1985.

[101] American Veterinary Medical Association, *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition*, 2013, https://www.avma.org/KB/Policies/Documents/euthanasia.pdf, accessed on November 21, 2013.

[102] National Research Council (US) Committee on Recognition and Alleviation of Pain in Laboratory Animals, 2009, *Recognition and Alleviation of Pain in Laboratory Animals*, Washington, DC: National Academies Press (US).

1475

1476 **APPENDIX B: TYPES OF ANIMAL CARE INTERVENTIONS**
1477
1478 As described in this guidance, animal care interventions incorporated into animal studies are
1479 divided into three categories based on the rationale for their use: (1) intervention as part of
1480 adequate veterinary care, (2) intervention to permit the manifestation of the disease or condition
1481 for the purpose of model development, and (3) intervention as supportive care to mimic the
1482 human clinical scenario. These categories of interventions are discussed here:
1483
1484 *Intervention as part of adequate veterinary care*: Animal studies conducted in the United States
1485 and its territories must comply with applicable laws and regulations as prescribed by the Animal
1486 Welfare Act[103] and the Public Health Service Policy on Humane Care and Use of Laboratory
1487 Animals.[104] In addition, all studies should comply with general principles for the care and use of
1488 animals in biomedical research (see Appendix A for details). Compliance with these laws and
1489 general principles ensures that adequate veterinary care is provided, such that animals
1490 experiencing more than momentary or slight pain or distress are provided relief through
1491 appropriate analgesia, treatment, or, when prospectively defined criteria are met, euthanasia.
1492 Exceptions to this standard are permitted only when scientifically justified and approved by the
1493 Institutional Animal Care and Use Committee. The standards for adequate veterinary care also
1494 include treatment of unexpected events, such as injury or the development of an unrelated
1495 disease. An example of an intervention that is considered part of adequate veterinary care is the
1496 administration of analgesics in a study assessing the effects of an investigational drug on
1497 vesicant-induced effects on the skin.
1498
1499 *Intervention to permit the manifestation of the disease or condition for the purpose of model*
1500 *development*: To study certain diseases or conditions, interventions are needed to permit the
1501 manifestation of the disease or condition of interest. Interventions used in this way are essential
1502 parts of the model development. For example, to establish a model of the gastrointestinal
1503 subsyndrome of acute radiation syndrome (GI-ARS), it is necessary to attenuate the potentially
1504 lethal effects of the hematologic subsyndrome of acute radiation syndrome (H-ARS) that occur
1505 before, or concomitantly with, GI-ARS. The interventions used to attenuate the H-ARS (e.g.,
1506 partial bone marrow shielding during irradiation or bone marrow transplantation) are considered
1507 to be components of model development.
1508
1509 *Intervention as supportive care to mimic the human clinical scenario*: Supportive care, as
1510 defined in this document, is needed only to mimic, to the extent possible, the human clinical
1511 scenario.[105] In general, it is relevant only for efficacy studies designed to support treatment of
1512 the disease or condition and the natural history studies on which the animal model is based.

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[103] See 7 U.S.C. 2131 et seq.

[104]National Institutes of Health, Office of Animal Welfare, "Public Health Service Policy on Humane Care and Use of Laboratory Animals," 2002, http://grants.nih.gov/grants/olaw/references/PHSPolicyLabAnimals.pdf, accessed on November 21, 2013.

[105] The need for supportive care should be directed by the concept of operations (i.e., how the product will be used during an incident).

1513   Animal supportive care can range from minimal intervention (particularly in the case of small
1514   rodents) to comprehensive medical support; however, it is not necessarily equal to patient care in
1515   a human clinical setting and in many cases may be significantly less intensive. The ability to
1516   provide certain types of supportive care may be species dependent (e.g., the ability to provide
1517   blood transfusions in a nonhuman primate model versus a rodent model). When included in an
1518   animal efficacy study, supportive care ideally should reflect the intended conditions of use of the
1519   investigational drug. It also should reflect the intended types of medical intervention and the
1520   timing of the availability of medical intervention expected in the human clinical or incident
1521   setting. The anticipated supportive care should be adapted, as appropriate, from the standard of
1522   human clinical practice to the animal species used, such as modifying the doses, route of
1523   administration, or the specific medical products administered.
1524
1525   When supportive care is administered to the animals as part of the design of the efficacy study,
1526   the study should show that the investigational drug with supportive care is superior to placebo
1527   with supportive care. When incorporated into a study, supportive care should be administered
1528   either to all animals on a set schedule or to individual animals according to prospectively defined
1529   triggers, based on preliminary studies or available literature. When supportive care will be
1530   administered to individual animals based on prospectively designed treatment triggers, the
1531   statistical plan should take into account the potential impact on the efficacy endpoint of differing
1532   supportive care among animals. The potential effects of the supportive care on the animal and
1533   on the PK and/or PD of the investigational drug should be considered in the design and
1534   interpretation of the study.
1535
1536
1537

1538 **APPENDIX C: GENERAL EXPECTATIONS FOR NATURAL HISTORY STUDIES**
1539
1540 *Natural history studies* are studies in which animals are exposed to a challenge agent and
1541 monitored to gain an understanding of the development and progression of the resulting disease
1542 or condition, including parameters such as time from exposure to onset of the manifestations,
1543 time course of the progression, severity, and manifestations (e.g., signs, clinical and pathological
1544 features, laboratory parameters, extent of organ involvement, morbidity, and outcome). Ideally,
1545 natural history studies should be prospectively designed,[106] adequately controlled, well-
1546 documented, and statistically powered to demonstrate the anticipated morbidity or mortality. In
1547 addition, the studies should include a statistical analysis of potential treatment triggers or critical
1548 determinants of disease or condition such as signs, endpoints, or biomarkers. Challenge dose
1549 standardization should occur before, or as part of, the natural history study.
1550
1551 In general, natural history studies should include randomized concurrent controls (i.e.,
1552 unchallenged control animals) to reduce experimental bias (e.g., age- and sex-matched controls,
1553 or controlling for the effect of vehicle on the respiratory tract of experimental animals in aerosol
1554 challenge models). Blinding should be used, to the extent possible, to reduce investigator bias.
1555 Observation times and/or frequencies should be specified in the study protocol and should be
1556 based on available information and/or preliminary studies. The frequency of observation should
1557 be adequate to characterize the course of disease or injury and to define the desired endpoints
1558 and treatment triggers. The frequency of observation may vary over the course of the study,
1559 depending on the actual mechanism of disease or injury. Observation frequency should be
1560 increased around the expected time of major morbidity or death to ensure animal welfare as well
1561 as to minimize the potential loss or compromise of data. Findings from the natural history
1562 studies should be substantiated through replication of the study or a demonstration of results
1563 consistent with other relevant studies. For example, the median survival at a relevant time point
1564 and time to the development of neutropenia following exposure to a specified dose of whole
1565 body radiation should be similar for irradiated rhesus macaques in the natural history studies and
1566 in the control groups for the associated efficacy studies.
1567
1568 The natural history studies should be adequate in design, conduct, and reporting. These studies,
1569 designated for drug development under the Animal Rule, will be subject to inspection and audit
1570 by FDA to verify the reliability of the data. The expectations for data quality and integrity for
1571 model-defining natural history studies submitted for qualification are discussed in section IV.B.
1572
1573 The general expectations with regard to the animals used in the investigation, study conduct, the
1574 study report, and the submission of the data and report are discussed in section IV.
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[106] When it is anticipated that supportive care will be used in the adequate and well-controlled animal efficacy
studies, the assessment of similar supportive care in model development, including the natural history studies used
to define the model, should be discussed with the review division (see section VI.A and Appendix B).

1576		**APPENDIX D: ACRONYMS AND ABBREVIATIONS**
1577		
1578	ADME	Absorption, distribution, metabolism, and excretion
1579	AMQP	Animal Model Qualification Program
1580	AUC	Area under the plasma concentration-time curve
1581	BARDA	Biomedical Advanced Research and Development Authority
1582	BLA	Biologics license application
1583	BPCA	Best Pharmaceuticals for Children Act
1584	BSL	Biosafety level
1585	CBER	Center for Biologics Evaluation and Research
1586	CDC	Centers for Disease Control and Prevention
1587	CDER	Center for Drug Evaluation and Research
1588	CFR	Code of Federal Regulations
1589	Cmax	Maximum (peak) plasma drug concentration
1590	Cmin	Minimum (trough) plasma drug concentration
1591	Css	Steady-state plasma concentration
1592	COU	Context of use
1593	eCTD	Electronic common technical document
1594	CYP450	Cytochrome P450
1595	DDT	Drug development tools
1596	E/R	Exposure-response
1597	EUA	Emergency use authorization
1598	FDA	U.S. Food and Drug Administration
1599	FD&C Act	Federal Food, Drug, and Cosmetic Act
1600	GI-ARS	Gastrointestinal subsyndrome of acute radiation syndrome
1601	GLP	Good laboratory practice regulations
1602	H-ARS	Hematopoietic subsyndrome of acute radiation syndrome
1603	HHS	Department of Health and Human Services
1604	IACUC	Institutional Animal Care and Use Committee
1605	IND	Investigational new drug
1606	LD_{50}	Lethal dose sufficient to kill 50% of those exposed to the agent
1607	MCMi	Medical Countermeasures initiative
1608	MIC	Minimum inhibitory concentration

1609	NDA	New drug application
1610	PBPK	Physiologically-based pharmacokinetic
1611	PD	Pharmacodynamic
1612	PK	Pharmacokinetic
1613	PREA	Pediatric Research Equity Act of 2003
1614	SPA	Special protocol assessment
1615	SNS	Strategic National Stockpile
1616	USC	United States Code